biologia plantarum

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Академик БОГУМИЛ НЕМЕЦ в полном здоровье и расцвете научной деятельности праздновал вместе со своими многочисленными учениками и сотрудниками 12 марта 1961 года **88-летие**

Academician BOHUMIL NĚMEC celebrated his **88th** birthday on March 12, 1961 in full mental and physical agility, in the circle of his numerous students and co-workers

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BIOLOGIA PLANTARUM (PRAHA)

3 (2) : 85—88, 1961

The Length of the First Leaf-Blade of Winter-Wheat is not an Indicator of Vernalization

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Received June 1, 1960

Délka čepele prvního listu ozimé pšenice není indikátorem jarovizace

Po inhibici jarovizace azidem sodným byla u ozimé pšenice sledována délka čepele prvního listu. Srovnáním různých variant a jarní odrůdy se ukázalo, že zkrácení délky čepele prvního listu nemá přímý vztah k jarovizaci.

Summary

The length of the leaf-blade of the first leaf of winter-wheat was investigated following inhibition of vernalization by sodium azide. It was shown by comparing different variants and the spring-wheat that the shortening of the leaf-blade in question is not directly related to vernalization.

Introduction

It was shown in a number of older papers that a decrease in length of the first leaf-blade represents one of the characteristic morphological features of vernalization (CHOLODNYJ 1936, THIMANN and LANE 1938, DENFFER 1939, MAEDA 1959). Cholodnyj used this phenomenon as a basis for his hormonal theory of the mechanism of vernalization. CHAILAKHYAN and ZHDANOVA (1938) maintain, on the contrary, that we are not dealing here with a specific effect of lower temperatures which may be encountered even in ecotypes without

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vernalization requirement. Recently, the topic was again taken up by HÄNSEL (1953, 1959) and PURVIS and HATCHER (1959). In his first paper, HÄNSEL (1953) supplies evidence for a close relationship between the degree of vernalization and the length of the blades of the first and second leaf. In his later paper (1959) he uses more caution when he considers the shortening of the leaf-blade as a consequence of the so-called "Kälte-metabolismus", i.e. of a non-specific (from the point of view of vernalization) effect of low temperatures. PURVIS and HATCHER then demonstrate quite convincingly in their de-vernalization experiments that we are dealing here with a non-specific morphological indicator of vernalization. In studying the physiology of vernalization, several results were obtained here which deal with the problems mentioned above and which are summarized in this paper.

Material and Methods

The winter-wheat *Triticum aestivum* L. of the sort Hodonínská holice and the spring-wheat *T. aestivum* L. of the sort Niva were used in the experiments. All the material represented the offspring of a single plant.

In 1958, the individual variants were vernalized following application of azide which was applied in an aqueous solution and used directly for soaking of the grains. In the winter sort sodium azide acted as an inhibitor of vernalization (on the application of azide for vernalization inhibition see KREKULE 1960) without affecting the rate of development of the spring sort. The length of the blade of the first leaf was then investigated in these variants.

Vernalization took place at $+0{\cdot}8° \pm 0{\cdot}3°$ C for 35 days. After the end of vernalization the grains were washed for 6 hours in running tap water. Seedlings representing an average of the given variant were then transferred (in lots of 10 plants) into flowerpots about 16 cm. in diameter and grown further under artificial illumination (a 16-hour photoperiod) in a green-house at temperatures above 15° C. The length of the blade of the first leaf was measured at the time of appearance of the fourth leaf and the size of the first leaf-blade did not change any more. The experiment was evaluated 75 days after vernalization by a morphological analysis of the growing points. The points were classified into 8 groups; some of them are defined here for illustration: 1 — a simple growing point, 2 — an elongating growing point, 2·5 — rudiments of ears may be seen, 3 — clear-cut primordia of ears, 6 — differentiation of stamens, 8 — earing.

In order to determine the degree of inhibition due to azide the respiratory activity of the grains was estimated several times in the course of vernalization by means of the Warburg manometric method.

Results and Discussion

It may be seen from Table 1 that in the concentration range of 5×10^{-1}M to 8×10^{-4}M azide completely blocked the vernalization process in the Hodonínská holice and the plants remained in the vegetative stage like the non-vernalized control. The rate of development of the spring sort was not affected by the inhibition. It further follows from the Table that the leaf-blade of the winter-wheat was shortened both in the vernalized control and in the variants inhibited by 8×10^{-4}M and 5×10^{-4}M azide by approximately the same amount. The differences between the vernalized control and the 1×10^{-4}M variant are also insignificant. It remains to be explained why

there is a significant difference between the variant inhibited by 8×10^{-4}M and that inhibited by 1×10^{-4}M sodium azide. It should be noted at this point that this difference could not be demonstrated on repeating this experiment. A similarly marked degree of shortening of the leaf-blade due to low temperature can be observed in the spring-wheat Niva, the inhibitors exhibiting here no effect on the rate of development. The application of inhibitors itself did not bring about any significant differences in the length of the blade (compare the variants N 8×10^{-4}M and N 1×10^{-4}M with the variants NKV).

Table 1

Variant	Growing point phase $\bar{x} \pm 3s_{\bar{x}}$	Length of axis in cm. $\bar{x} \pm 3s_{\bar{x}}$	Number of plants	Length of leaf-blade of 1st leaf in mm. $\bar{x} \pm 3s_{\bar{x}}$	%
Hh 8×10^{-4}	$1{\cdot}1 \pm 3 . 0{\cdot}09$	—	11	$55 \pm 3 . 3{\cdot}9$	65,5
Hh 5×10^{-4}	$1{\cdot}0 \pm 3 . 0{\cdot}0$	—	10	$49 \pm 3 . 6{\cdot}3$	58·5
Hh 1×10^{-4}	$5{\cdot}4 \pm 3 . 0{\cdot}23$	$9{\cdot}8 \pm 3 . 1{\cdot}80$	8	$41 \pm 3 . 4{\cdot}1$	49·0
Hh KV	$6{\cdot}7 \pm 3 . 0{\cdot}24$	$16{\cdot}8 \pm 3 . 1{\cdot}67$	9	$50 \pm 3 . 2{\cdot}9$	59,5
Hh K	$1{\cdot}2 \pm 3 . 0{\cdot}15$	—	12	$84 \pm 3 . 4{\cdot}5$	100·0
N 8×10^{-4}	earing	118·0	15	$76 \pm 3 . 5{\cdot}9$	72·5
N 1×10^{-4}	earing	118·0	14	$65 \pm 3 . 6{\cdot}8$	62·0
N KV	earing	118·0	18	$70 \pm 3 . 5{\cdot}2$	66·5
N K	earing	118·0	14	$105 \pm 3 . 2{\cdot}9$	100·0

Legend: Hh — Hodonínská holice, winter-wheat. N — Niva, spring wheat. The column of variants includes molar concentrations of sodium azide. K — non-vernalized control. KV — vernalized control. Maximum difference at the time of earing between the Niva variants was two days. The last column (%) refers to the length of the leaf-blade of the non-vernalized control.

All the data given above show that it is not possible to employ the shortening of the leaf-blade of the first leaf as an indicator of vernalization of winter cereals. The phenomenon appears to be associated quite unspecifically with the effect of low temperature. This, however, does not exclude the possibility of a correlation of the two parameters under common vernalization conditions as suggested e.g. by HÄNSEL (1953).

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Длина пластинки первого листа озимой пшеницы не является индикатором яровизации

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При ингибиции яровизации у озимой пшеницы азидом натрия была исследована длина листовой пластинки первого листа. При сравнении разных вариантов с яровой пшеницей оказалось, что укорочение листовой пластинки не имеет прямого отношения к яровизации.

BIOLOGIA PLANTARUM (PRAHA)

3 (2) : 89—98, 1961

Untersuchungen über die Wirkung einiger 1,2,4-Triazolderivate auf das Wachstum der Keimlinge von *Sinapis alba* L.

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Eingegangen am 8. Juli 1960

Studie o působení některých derivátů 1,2,4-triazolu na růst klíčních rostlin *Sinapis alba* L.

Sledováním fysiologických účinků dvanácti dosud nezkoušených látek — devíti derivátů 1,2,4-triazolu III až XI, po jedné ze sloučenin řady 1,3,4-oxadiazolové XII a tetrazolové XIII a konečně N-(2-thiazolyl)-amidu kyseliny nikotinové (XVIII) — na klíční rostliny *Sinapis alba* L. bylo zjištěno: Ani jedna ze studovaných sloučenin nepůsobí herbicidně. Deriváty V a VIII naproti tomu zřetelně stimulují růst pokusných rostlin. Na základě dalších zjištění lze důvodně předpokládat, že herbicidní účinnost 3-amino-1,2,4-triazolu (II) je úzce spjata s konstitucí molekuly a že v tomto případě je nezbytné, aby substituentem na triazolovém seskupení byla aminoskupina, a to ještě výhradně v poloze 3. Nalezené výsledky jsou v souladu s teorií, jež příčinu silného herbicidního účinku 3-aminotriazolu (II) připisuje komplexotvorné schopnosti molekuly. U známých stimulátorů růstu XIV až XVI, použitých v dávce 100 mg v litru živného roztoku (koncentrace 10^{-1}), stimulační účinek se změnil v herbicidní.

Zusammenfassung

Es wurde die Wirkung zwölf bis jetzt noch nicht auf die Wachstumsaktivität der Keimlinge von *Sinapis alba* L. geprüfter Substanzen untersucht. Neun Stoffe von diesen waren 1,2,4-Triazolderivate III bis XI, je ein Vertreter der 1,3,4-Oxa-diazolreihe XII und der Tetrazolreihe XIII. Substanz Nummer XVIII war ein N-substituiertes Nikotinsäureamid. Bei diesen Untersuchungen wurden folgende Ergebnisse erzielt:

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1. Keine der untersuchten Substanzen zeigte eine durchschlagend hemmende Wirkung.

2. Die Derivate V und VIII wirkten im Gegenteil wachstumsfördernd auf Wurzeln und Hypokotyle der Versuchspflanzen.

3. Diese Untersuchungen scheinen zu begründen, dass die herbizide Wirkung des 3-Amino-1,2,4- triazols (II) mit der Konstitution seines Moleküls gegeben ist und dass in diesem Falle der Substituent am Triazolringe eine Aminogruppe unbedingt in der Lage 3 sein muss.

4. Die gefundenen Ergebnisse bestätigen die Theorie, dass die herbizide Wirkung des 3-Aminotriazols (II) durch die Komplexfähigkeit seines Moleküls hervorgerufen wird.

5. Bei den bekannten Wuchsstoffen XIV bis XVI zeigt sich, vorausgesetzt, dass sie in der gleichen Konzentration wie die geprüften Stoffe, d. h. 100 mg auf einen Liter Nährstofflösung (Konzentration 10^{-1}), und auch sonst unter gleichen Bedingungen angewendet werden, eine stark hemmende Wirkung.

Einleitung

Zu dieser Arbeit wurden wir durch die Tatsache veranlasst, dass das Hydrazid der Isonikotinsäure (I), das als Antituberkulotikum benützt wird (Novotný 1957 und 1959) das Pflanzenwachstum stark hemmt (Norman 1956). Dies ist auch aus dem Grunde beachtenswert, weil das Amid der isomeren Nikotinsäure (XVII), eine chemisch sehr verwandte Verbindung, im Gegensatz eine wachstumsfördernde Substanz für die Pflanzen ist. Wir hielten es deswegen für zweckmässig, die physiologische Wirkung bei einigen Verbindungen der 1,2,4-Triazolreihe auf die Pflanzen zu verfolgen, die zur Untersuchung ihrer antituberkulotischen Eigenschaften synthetisiert worden waren. Die Berechtigung zur Voraussetzung, dass auch diese Stoffe herbizide Wirkung haben können, sahen wir darin, dass sie im Grunde das gleiche heterocyklische Skelet wie das Molekül des 3-Aminotriazols besitzen, das ein systemisches Unkrautbekämpfungsmittel mit einer hohen Phytotoxizität ist und spezifische Symptome hervorruft (Hauser und Thompson 1954; Hall, Johnson und Leinweber 1954).

Ausserdem haben wir im voraus auch die Möglichkeit nicht ausgeschlossen, dass die genannten Derivate des 1,2,4-Triazols das Wachstum fördern, dass also diese Derivate dem 3-Aminotriazol (II) in antagonistischer Wirkung gegenüberstehen, ähnlich wie das Hydrazid der Isonikotinsäure (I) und das Nikotinsäureamid (XVII). Die meisten der geprüften 1,2,4-Triazolderivate III bis IX unterscheiden sich vom 3-Aminotriazol (II) durch die Lage der Aminogruppe, die übrigen X und XI besitzen sie überhaupt nicht.

Neben der Erforschung der eigentlichen Wirkung auf das Wachstum der Versuchspflanzen stellten wir uns deshalb auch die Aufgabe, zu ergründen, ob eine solche grundsätzliche Änderung der Struktur auch eine Änderung der Wirkung zur Folge hat, und welche Reaktion die Elliminierung der Aminogruppe hervorruft. Endlich wollten wir auch feststellen, ob sich die Wirkung

bei einzelnen Vertretern der steigenden homologen Reihen ändert, und zweitens, ob die analogen Verbindungen, bei denen nur die ursprüngliche Funktionsgruppe ausgewechselt wurde, eine bedeutende Änderung der Wirkung erleiden.

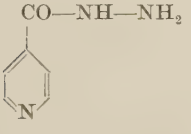
Geprüfte Substanzen

In dieser Arbeit haben wir die Wirkung zwölf bis jetzt noch nicht untersuchter Verbindungen verfolgt, wobei wir 3-Amino-1,2,4-triazol (II) als Vergleichspräparat benützten. Neun von den geprüften Stoffen sind Derivate des 1,2,4-Triazols. Zum Unterschied von 3-Aminotriazol (II), das eine Aminogruppe mit der Bindung in der Lage 3 aufweist, ist bei sieben dieser Verbindungen die Aminogruppe in der Lage 4 eingebaut. Es wurden folgende Stoffe untersucht:

4-Amino-1,2,4-triazol (III), F 81—82° *)
4-Amino-3,5-dimethyl-1,2,4-triazol (IV), F 197—198°
4-Amino-3,5-diethyl-1,2,4-triazol (V), F 165—166°
4-Amino-3,5-dioxy-1,2,4-triazol (VI), F 267—268° (Zers.)
4-Amino-3,5-bis-oxymethyl-1,2,4-triazol (VII), F 207—208°
4-Amino-3,5-bis-(α-oxyethyl)-1,2,4-triazol (VIII), F 164—167°
4-Amino-3,5-bis-(β-oxyethyl)-1,2,4-triazol (IX), F 151°

Die weiteren zwei Stoffe sind Derivate des 1,2,4-Triazols, denen die Aminogruppe völlig fehlt; die letzte Verbindung endlich ist ein 1,3,4-Oxa-diazolderivat:

3,5-Dioxy-1,2,4-triazol (X), F 242—243°
5-Sulfhydro-3-oxy-1,2,4-triazol (XI), F 177°
2-(4′-Pyridyl)-1,3,4-oxa-diazol (XII), F 120—121°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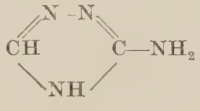
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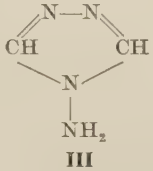
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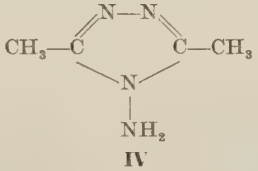
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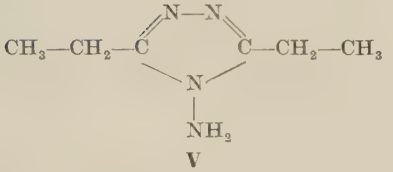
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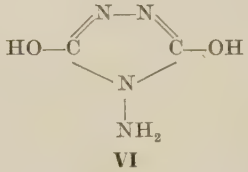
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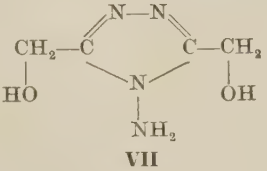
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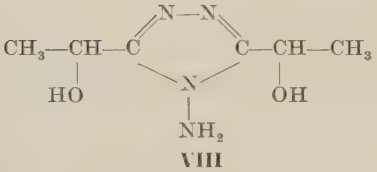
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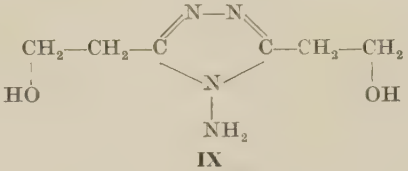
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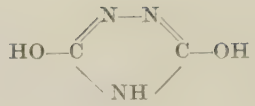

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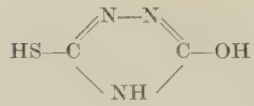

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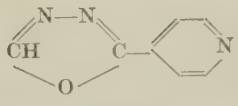

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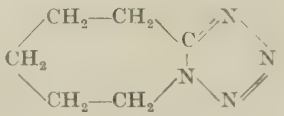
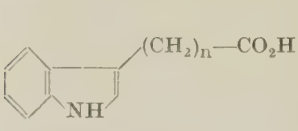


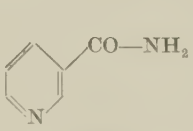
XIII

Ausser den genannten 1,2,4-Triazolderivaten wurde auch eine Verbindung der Tetrazolreihe geprüft, und zwar Pentamethylentetrazol (Pentazol-Spofa, XIII). Der zwölfte Stoff ist das weiter genannte N-substituierte Nikotinsäureamid XVIII.

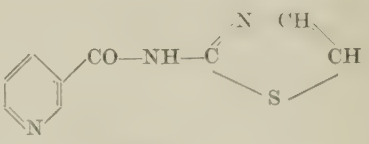
Die Voraussetzung, dass einige der geprüften Stoffe fördernd auf das Wachstum wirken können, veranlasste uns unter gleichen Bedingungen auch bekannte Wachstums-Stimulatoren, und zwar 3-Indolylessigsäure (XIV), β-(3-Indolyl)-propionsäure (XV) und γ-(3-Indolyl)-buttersäure (XVI) zu prüfen. Zur Vollständigkeit wurde diese Reihe von Wuchsstoffen noch durch Nikotinsäureamid (XVII) und durch das in der Literatur noch nicht beschriebene N-(2-Thiazolyl)-nikotinsäureamid (XVIII) ergänzt; dieses wurde vom zweiten Autor dieser Arbeit im Jahre 1958 synthetisiert*) und seine Wirkung auf das Pflanzenwachstum wurde in der vorliegenden Untersuchung zum erstenmal geprüft.



XIV n = 1
XV n = 2
XVI n = 3



XVII



XVIII

Die geprüften Substanzen und die Vergleichsstoffe wurden in einer Konzentration von 10^{-1} (100 mg im Liter) in van der Crones Nährstofflösung mit Zusatz von AZ-Lösung gelöst, wobei als Kontrolle reine Nährstofflösung nach van der Crone diente. Bei vorläufigen Orientierungsversuchen wurde auch die Konzentration 10^{-5} (0,01 mg im Liter) und 10^{-3} (1 mg im Liter) geprüft.

Methodik

1. Methode zur Verfolgung der toxischen Wirkung auf die Pflanzenzelle. Auf Grund der chemischen Konstitution der geprüften Triazolderivate, die dem 3-Aminotriazol (II) ähnelt, war zu erwarten, dass auch der Mechanismus ihrer Wirkung ähnlich dem Mechanismus der

*) F 212—214°.

Elementaranalyse: berechnet 52,67% C, 3,44% H, 20,48% N, 15,62% S
gefunden 52,89% C, 3,50% H, 20,32% N, 15,28% S.

Wirkung des 3-Aminotriazols sei, das heisst, dass sie systemische Herbizide ohne unmittelbare Wirkung auf die Pflanzenzelle sein werden. Um sich von der Richtigkeit dieser Voraussetzung zu überzeugen, untersuchten wir die unmittelbare toxische Wirkung der neun geprüften Stoffe und auch der Vergleichsstoffe nach Art und Dauer der Plasmabewegungsstörung der inneren Epidermiszellen der Zwiebelschuppe von *Allium cepa* L. (STRUGGER 1949, MAYER 1957). Das Absterben der Zellen haben wir nicht nur wie STRUGGER durch die Verfolgung der Plasmabewegung und Brownschen Molekularbewegung und die Fähigkeit der lebenden Zellen zu plasmolysieren festgestellt, sondern auch durch Färbung der abgestorbenen Zellen mit Methylenblau.

Von einer frischen Zwiebelschuppe wurden die Epidermiszellen abgetrennt und in 5 × 5 mm grosse Stückchen zerschnitten. Ein Teil wurde auf sechs Stunden in reine Nährstofflösung eingetaucht, die anderen in die Nährstofflösung mit Zusatz der geprüften Substanz in der Konzentration 10^{-1}. In bestimmten Zeitabschnitten wurde die Plasmabewegung mikroskopisch bei 900× Vergrösserung mit Hilfe des Phasenkontrastes beobachtet. Dann wurde jedes Präparat mit 0,6 M-Kaliumnitrat plasmolysiert. Durch Färbung mit Methylenblau wurden die gewonnenen Resultate noch einmal bestätigt. Nach dieser Vervollständigung ergab die Methode so genaue Resultate über die Identifizierung der abgestorbenen Zellen, dass wir uns bei unseren Untersuchungen auf sie beschränken konnten.

2. Methode zur Verfolgung der Wachstumsaktivität. Die Unterscheidung zwischen den totalen und den selektiven Unkrautbekämpfungsmitteln liegt in der Art ihrer physiologischen Wirkung auf die Pflanze. Ein selektives (systemisches) Bekämpfungsmittel wirkt auf den Stoffwechsel der Pflanzen ein und verursacht spezifische Änderungen im Metabolismus (WOODFORD 1958). Deshalb ist die Wirkung eines herbiziden Stoffes viel langsamer und macht sich äusserlich in erster Reihe durch die Hemmung des Wachstums erkennbar. Aus diesem Grunde beurteilen wir die Wirkung der geprüften Substanzen und der Vergleichstoffe unter Anwendung einer biologischen Methode für wachstumregulierende Substanzen nach AUDUS. Wir benützen aber als Testpflanze *Sinapis alba* L., die in der Nährstofflösung van der Crones gezüchtet wurde; auch mussten wir die Methode in der Art der Kultivierung unseren Bedingungen anpassen. Das Filtrierpapier (AUDUS 1951) oder der Glasfaserwebstoff (AUDUS und QUASTEL 1947), der in Rollen gedreht in die Kulturgefässe eingeschoben wird, um die Nährstofflösung für die Wurzeln, die zwischen diesem Material und der Wand des Glasgefässes wachsen, aufzusaugen, ist für länger dauernde Versuche ungeeignet. Das Filtrierpapier, das von der Nährstofflösung immer nass bleibt, ist ein sehr gutes Nährmedium für unerwünschte Pilze. Der Nachteil des Glasfaserwebstoffes liegt in seiner Steifheit und in der verhältnismässig geringen Saugkraft. Aus dem Material, das für uns erreichbar war, hat sich dichter Flanell am besten bewährt. Absichtlich hatten wir auch die vorherige Sterilisierung mit Quecksilberchlorid weggelassen, da in unserem Falle die Gefahr drohte, dass es mit einigen der geprüften Substanzen zur Bildung eines Quecksilberkomplexes kommen könnte, der die Wirkung auf die Pflanzen beeinflussen würde. Die Sterilisierung der Samen und des Filtrierpapiers mit UV-Strahlen (Philips TUV-30 W), die am Anfang durchgeführt wurde, erwies sich als ungeeignet. Die Strahlen beeinflussen nämlich das Keimen und auch das Wachstum in negativem Sinne, wobei der Angriff der Schimmelpilze nur um zwei Tage verspätet wurde. Bei unseren Vorversuchen haben wir beobachtet, dass sich unerwünschte Schimmelpilze und Bakterien hauptsächlich von der abgestorbenen Samendecke aus weiter verbreiten. Aus diesem Grunde begnügten wir uns mit der Sterilisierung der Gefässe und die Samendecken der Sämmlinge haben wir gleich am zweiten Tag beseitigt. Wir verbesserten diese Methode noch dadurch, dass wir nicht nur das Wachstum der Wurzeln, sondern auch das der Hypokotylen verfolgten.

Im Laufe der Vorversuche haben wir den Zuwachs der behandelten und der Kontrollpflanzen zwanzig Tage lang gemessen. Da aber festgestellt wurde, dass die Hemmung oder die Förderung des Pflanzenwachstums sich bereits in den ersten Tagen bemerkbar macht, wurden die Messungen bei den eigentlichen Versuchen nur sieben Tage lang durchgeführt. Dann wurden die Pflanzen noch zehn bis vierzehn Tage weiter beobachtet, ob sich an dem gewonnenen Resultat etwas verändert hatte. Die Samen von *Sinapis alba* L. haben wir in Keimgefässen, die mit van der Crone Nährstofflösung mit AZ-Zusatzlösung (Kontrolle) (SCHROPP 1951) und endlich mit den untersuchten Substanzen in entsprechender Konzentration gefüllt waren, vorgekeimt. Nach 24 Stunden, wenn der Keim ungefähr 1 bis 2 mm Länge erreicht hatte, wurden die keimenden Samen in Kultureprouvetten (innerer Durchschnitt 22 mm, Länge 170 mm) übertragen. In die Eprouvetten wurde vorher ein Streifen Flanell (75 mm × 135 mm) zylinderförmig längs der inneren Wand eingeschoben und 20 ml Lösung nachgefüllt. Wenn der Flanell ganz feucht war, wurden zwischen seinen oberen Rand und die Wand der Eprouvette je acht Samen eingelegt. Die Wirkung der geprüften Substanzen wurde in den eigentlichen

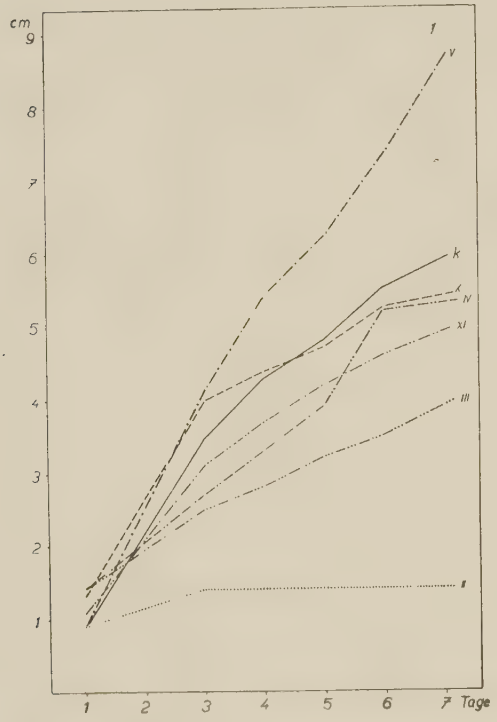
Untersuchungen an 80 Keimlingen und in den Vorversuchen annähernd an 100 Keimlingen beobachtet. Die Eprouvetten wurden leicht mit Watte verschlossen, um das Verdunsten der Nährstofflösung und Infektion der ganz kleinen Keimlinge zu verhindern; schliesslich wurden die Kulturgefässe in eine Rolle schwarzen Papiers bis in die Höhe der Samen eingeschoben und in Ständern auf dem Vegetationstisch untergebracht. Hier wurden die Versuchspflanzen täglich zehn Stunden lang mit acht Leuchtröhren (je 40 W, 120 cm lang), die in 35 cm Entfernung von den Pflanzen angebracht werden, beleuchtet. Die Lichtintensität betrug 3600 Lux, die Leuchtröhren schalteten sich automatisch ein und aus. Im Laufe der Untersuchungen wurde die Wärme bei 25° C gehalten und die Nährstofflösung wurde immer auf das ursprüngliche Volumen von 20 ml nachgefüllt. Unter gleichen Versuchsbedingungen wurde auch das Vorkeimen durchgeführt.

Ergebnisse und Diskussion

Die benützte Modifikation der AUDUS-schen Methode hat viele Vorteile. Die Kultivierung der Versuchspflanzen in Nährstofflösungen auf dem Vegetationstisch ist verlässlich reproduzierbar, da die Bedingungen, hauptsächlich die Intensität und die Zeit der Beleuchtung und die Wärme konstant gehalten werden können, was einen Vergleich und eine leichte Kontrolle der gewonnenen Resultate aus verschiedenen Versuchsserien ermöglicht. Das Messen der Wurzeln und der Hypokotylen ist sehr einfach (mit einem Millimeterpapierstreifen) und besonders im Falle der Hypokotylen auch sehr genau, da nur ein einziger Messwert zu beurteilen ist. Weniger genaue Messwerte bekommt man, wenn dagegen nur die Wurzeln gemessen werden, denn es kommt bei manchen Pflanzen der Versuchsserie oft zur Hemmung oder sogar zur Einstellung des Wachstums der Hauptwurzel, was dazu führt, dass viele Seitenwurzeln ausschiessen, deren Messung praktisch unmöglich ist. Wenn nur das Wachstum der Hauptwurzel beurteilt wird, zeigen die Kurven auf dem Diagramm eine grosse Streuung. Das eigentliche Wachstum des Hypokotyls der Pflanze wird aber nicht beeinflusst, denn die Seitenwurzeln genügen zur Ernährung der Pflanze. Die Ursache solcher ungenauen Messungen des Zuwachses der Hauptwurzel darf aber nicht als eine wachstumshemmende Wirkung der geprüften Substanz erklärt werden.

Die Resultate hinsichtlich der physiologischen Wirkung der geprüften Substanzen in der Konzentration 10^{-1} (100 mg pro Liter) stellen Durchschnittswerte von 80 gemessenen Pflanzen vor, die auf eine Pflanze umgerechnet wurden. Das Endergebniss wurde in fünf Versuchsserien bestätigt. Die vorherigen Untersuchungen mit der Konzentration 10^{-5} (0,01 mg im Liter) und 10^{-3} (1 mg im Liter) haben wir nicht angegeben. Die Resultate in den Diagrammen 1, 3 und 5 beziehen sich auf das Wurzelwachstum der Pflanzen, die mit 3-Aminotriazol (II) als Vergleichspräparat und mit den Substanzen III bis XVIII behandelt wurden und auf die entsprechende Kontrole. Die korrespondierenden Messwerte der Hypokotyle sind durch die Kurven in den Diagrammen 2, 4 und 6 dargestellt.

In keinem Falle haben wir ein Absterben der Versuchspflanzen beobachtet. Aus den gewonnenen Resultaten geht hervor, dass keine der geprüften Substanzen eine herbizide Wirkung besitzt. In der Konzentration 10^{-5} und 10^{-3} zeigt auch 3-Aminotriazol (II) keine hemmende Wirkung, vielmehr wurde das Wachstum leicht gefördert.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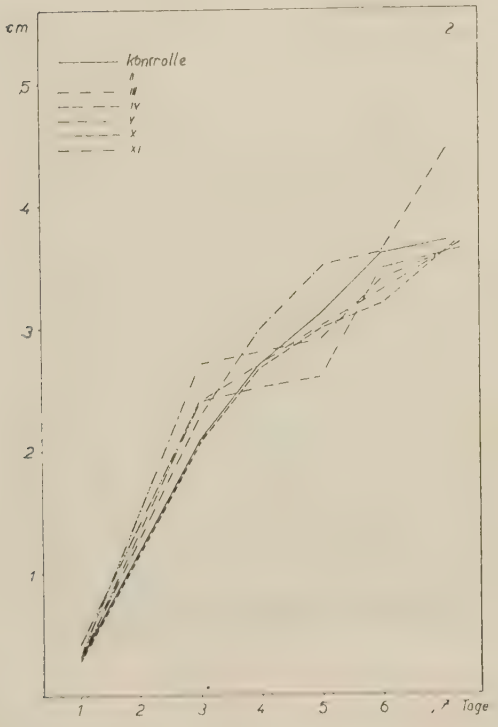
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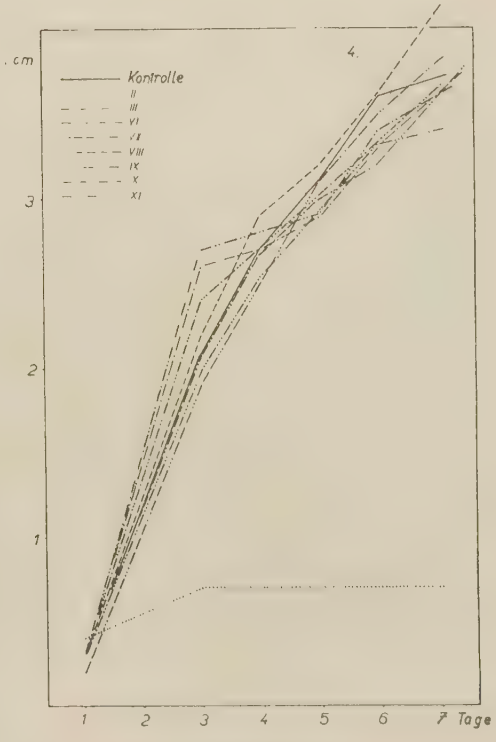
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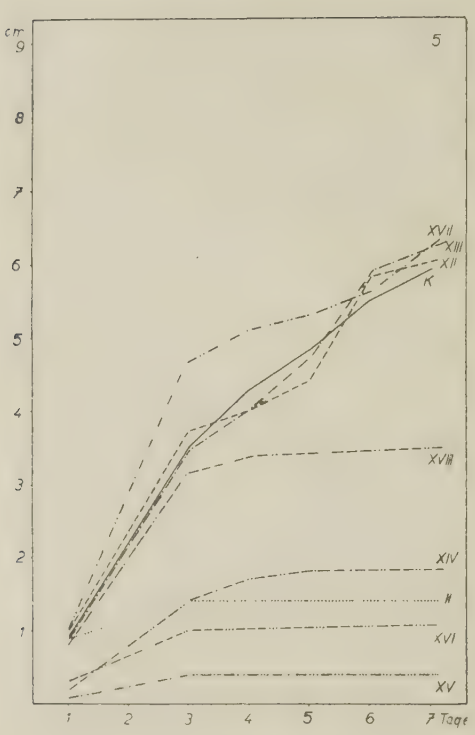

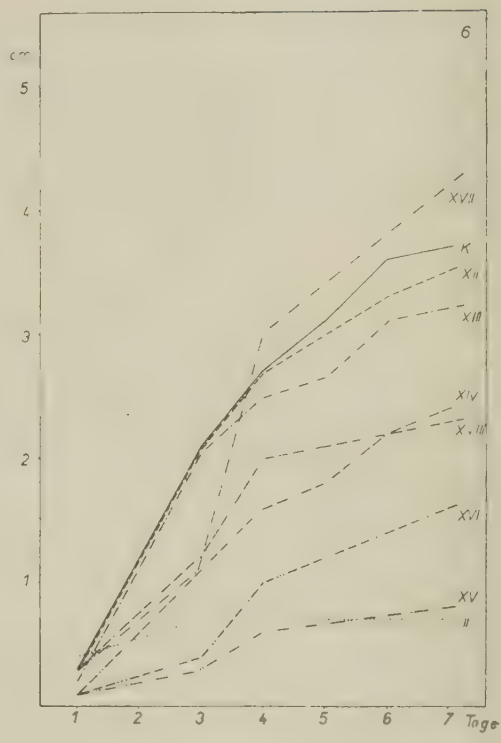

Dass keine hemmende Wirkung bei den 1,2,4-Triazolderivaten vorliegt, also Substanzen mit dem gleichen heterocyklischen Skelet wie es 3-Aminotriazol aufweisst, berechtigt unsere Voraussetzung, dass für eine herbizide Wirkung höchstwahrscheinlich eine Aminogruppe und zwar ausschliesslich in der Lage 3 nötig ist. Eine Hemmung des Wachstums der Hauptwurzel tritt bei einigen Versuchspflanzen in der Versuchsserie der Substanz III nur scheinbar ein, denn die Pflanze hat diese durch das Ausschiessen vieler Seitenwurzeln ersetzt, so dass das Hypokotylenwachstum nicht beeinflusst wurde. Das bestätigt unsere Behauptung, dass die Messwerte des Wurzelwachstums mit einem Fehler belastet sind, wie bereits oben erwähnt wurde.

Die Substanzen V und VIII zeigen im Gegenteil eine stimulierende Wirkung sowohl auf das Wurzelwachstum, als auch auf das Hypokotylenwachstum. Diese beide Substanzen fördern das Wachstum nicht nur in der Konzentration 10^{-5} und 10^{-3}, sondern auch in einer verhältnismässig hohen Konzentration 10^{-1}, bei der auch bekannte Heteroauxin-Wuchsstoffe, wie XIV bis XVI, das Wachstum hemmen. Dieses geht aus dem Kurvenverlauf in dem zugehörigen Diagramm 5 und 6 hervor. Nur Nikotinsäureamid (XVII) behielt auch in dieser Konzentration eine unzweifelhaft wachstumsfördernde Wirkung. Da diese beiden Verbindungen V und VIII die letzten Vertreter der homologen Reihen jener Substanzen sind, die wir zur Verfügung hatten, wäre es sehr interessant, die Wirkung noch höherer Homologen in beiden Reihen zu untersuchen.

Bei Vertretern der homologen Reihe III, IV und V oder III, VI, VII, VIII und IX kann man eine steigende Tendenz der Wirksamkeit mit zunehmendem Molekulargewicht beobachten. Die Unterschiede zwischen den einander folgenden Substanzen sind aber sehr gering. Dieses ist nicht überraschend, da der homologe Zuwachs nur eine geringe Veränderung bedeutet, wobei das Grundskelet des Moleküls nicht beeinflusst wird.

Die Substanzen X und XI weichen von einander nur durch ein Schwefelatom ab, das bei der Verbindung XI den Sauerstoff in der Hydroxylgruppe vertritt. Die Veränderung der Wirksamkeit ist auch hier nur unbedeutend.

Als tatsächliche Ursache der herbiziden Wirkung des 3-Aminotriazols (II), die sich deutlich nach unseren Untersuchungen in Nährstofflösungen schon in der Konzentration 10^{-2} (10 mg im Liter) bemerkbar machte, wird seine Neigung mit Metallionen Komplexe zu bilden gehalten (Sund 1956; Menoret und Tracez 1957). Durch die Bildung solch eines Komplexes mit dem Magnesiumion, das zum Aufbau des Chlorophylls in den jüngsten Entwicklungsstadien der Pflanzen nötig ist, und durch die Enfernung des Magnesiumions aus den bereits aufgebauten Chlorophyllmolekülen wird wahrscheinlich entweder die Chlorophyllbildung völlig unterbunden, oder eine Destruktion des Chlorophylls verursacht. Beide Vorgänge wirken hemmend auf die Assimilation, da der Katalysator fehlt. Bei unseren Untersuchungen zeigten sich die geprüften 1,2,4-Triazolderivate als wachstumshemmende Stoffe wirkungslos. Sechs von den benützten Verbindungen III, IV, V, IX, X und XI, aus deren Strukturformeln deduziert werden kann, dass sie keine Metallkomplexe bilden können, stellen die Bestätigung dieser Ansicht vor. Diese Tatsache ist im vollen Einklang mit unserer schon früher erwähnten Ansicht, dass die Triazolgruppierung eine Aminogruppe in der Lage 3 als Substituten besitzen muss, denn nur in diesem Falle kann sie mit einem Metallion einen Komplex bilden. Die übrigen drei Verbindungen VI bis VIII haben zwar auch Voraussetzungen zur Komplexbildung, es würde aber ein anderer Typus von Komplexen sein, wahrscheinlich auch mit anderen Eigenschaften, als die Komplexverbindung, die aus 3-Aminotriazol (II) entsteht. Den untersuchten Derivaten des 1,3,4-Oxa-diazols (XV) und Pentamethylentetrazols (XIII) fehlt die Komplexbildungfähigkeit völlig.

Keine der untersuchten Substanzen, einschliesslich 3-Aminotriazol (II), wirkt toxisch auf die Epidermiszellen von *Allium cepa* L. Die behandelten Zellen zeigen Plasmabewegungserscheinungen. Nach Einwirken von 0,6 M-Kaliumnitrat tritt in zehn Minuten Plasmolyse ein, die Zellen sind nicht mehr durch Methylenblau färbbar. Dies ist ein Beweis, dass die geprüften Substanzen für die Pflanzenzelle unschädlich sind. Unsere Beobachtungen sind im Einklang mit der schon früher erwähnten Ansicht von Woodford (Woodford 1958), dass systemische Unkrautbekämpfungsmittel nicht unmittelbar auf die Pflanzenzelle wirken, sondern den gesammten Metabolismus beeinflussen und seinen normalen Gang stören.

Es ist uns eine angenehme Pflicht Herren Dr. C. Blattný, Mitglied der Akademie, an dieser Stelle unseren Dank für sein Interesse, mit dem er unsere Arbeit verfolgte, auszusprechen. Auch danken wir Herren PhMr Ing Alois Novotný aus dem Forschungsinstitut für Pharmazie und Biochemie für die Freundlichkeit, mit der er uns die erforderlichen Substanzen III bis XIII zur Verfügung stellte, und unserer Mitarbeiterin R. Benišková für die genau durchgeführten Messungen der Versuchspflanzen während allen Untersuchungen.

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Ольга Маковцова, Павел Вацулик (Отдел фитопатологии растений Биологического института, ЧСАН, Прага)

Исследование влияния некоторых дериватов 1, 2, 4-триазола на рост всходящих растений *Sinapis álbá* L.

BIOLOGIA PLANTARUM (PRAHA) 3 (2) : 89—98, 1961

При рассмотрении физиологических влияний двенадцати не испытываемых до сих пор элементов — девяти дериватов 1, 2, 4 — триазола III—XI, по одному из соединений 1, 3, 4-оксадиазолового XII и тетразиалового ряда и, наконец, II — замещенного амида никотиновой кислоты — на всходящие растения *Sinapis alba* L., было установлено:

1. Ни одно из соединений не проявляет гербицидных действий.
2. Дериваты V и VIII ясно стимулируют рост подопытных растений.
3. Не без основания можно предполагать, что гербицидное действие 3-амино-1,2,4-триазола (АТА II) узко связано со строением молекулы и что в этом случае необходимо, чтобы субститует на триазоловой группе была аминогруппа, исключительно в положении 3.
4. Установленные результаты подтверждают теорию, что причиной сильного гербицидного влияния АТА (II) является комплексной способности молекулы.
5. У известных стимуляторов роста XIV—XVI, использованных в дозе 100 мг в литре питательного раствора, влияние стимуляции изменилось в слабогербицидное.

BIOLOGIA PLANTARUM (PRAHA)

3 (2) : 99—106, 1961

Formation of Secondary Xylem in Apricots (*Prunus armeniaca* L.) without the Participation of Buds

JIŘÍ DVOŘÁK

The Fruit Research Institute, Czechoslovak Academy of Agricultural Sciences, Holovousy*

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Vznik sekundárního xylemu u meruňky *(Prunus armeniaca* L.) bez účasti rašících pupenů

V předložené práci je sledována tvorba sekundárního xylemu u meruňky v závislosti na rašení pupenů. V odříznutých letorostech, u nichž byly odstraněny pupeny, se vytvářel sekundární xylem pouze při aplikaci kys. β-indolyloctové. U 1—3letých stromků s odstraněnými pupeny došlo k vytvoření xylemu v síle 1—2 vrstev tracheji pouze ve kmeni, nikoliv v letorostech a 2letých větvích. Při studiu průběhu tvorby xylemu u stromů meruněk různého stáří, při rašení listových pupenů bylo zjištěno, že nový xylem vzniká při rašení pupenů současně v letorostu pod pupenem a na basi kmene. Ve větvích 2—3letých a v letorostu na straně odvrácené od pupene se tvorba xylemu opožďuje. Tato zjištění odporují dosud uváděnému schématu, podle něhož u stromů dochází k tvorbě nového xylemu nejprve pod rašícím pupenem a pak se postupně tvorba xylemu šíří basipetálně až k basi kmene.

Summary

In experiments with cut-off shoots of apricots from which the buds were removed, normal xylem formed only when β indole acetic acid was applied at the sites from which the buds were removed. The newly formed xylem did not differ from xylem formed under the bursting buds.

In experiments with the disbudding of whole apricot trees, it was found that new xylem begins to form (one layer of trachea thick) even in trees with the buds removed. This xylem formed only in the stem especially at its base and not in the shoots. No abnormal tissue formation was detected in trees with buds removed.

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A study of the formation of new xylem in various parts of trees during the opening of leaf buds showed that new xylem forms simultaneously under the breaking buds in shoots and at the base of the stem. In two to three-year old branches xylem forms later.

Introduction

This work was begun to clear up several questions connected with pathohistological phenomena during the apoplexy of Apricots. Its aim was to determine cambial activity in trees which have all conditions for division except bursting buds.

It is known from older works beginning with HARTIG (1857), that cambium begins to divide and causes the formation of secondary xylem first of all under the buds and that this division gradually spreads basipetally right to the base of the stem and to the roots. (Information not given in the references is drawn from the review of the literature by LADEFOGED 1952). Isolated authors, for instance BROWN 1915 in *Pinus strobus* L., discovered that in the spring new xylem was formed first of all in the stem. KNUDSON 1916 asserts that during the developing of buds in peach trees, the formation of new xylem occurs simultaneously in one-, five-, and ten-year old branches. PRIESTLEY 1930, however, takes a critical stand against these findings and considers them erroneous due to incorrect experimental technique.

In trees with ring porous wood, LADEFOGED 1952 ascertained the formation of new wood in shoots at the base of the buds 1—9 days before their bursting. New xylem was formed in the stems of these trees at the time of the breaking of the buds or a few days earlier.

In trees with diffuse porous wood, the formation of new xylem under the buds occurred in five species at the same time or at the most, two days before the breaking of the buds. In four species (including *Prunus avium*), division of the cambium occurred up to one week before the breaking of the buds. New xylem was formed in the stem 7—43 days (15 days in *Prunus avium*) after the breaking of the buds. Apricot (*Prunus armeniaca* L.) is inclined to form ring porous wood, although the other woods of the Rosaceae family have diffuse porous wood.

Growth substances flow from the breaking buds which stimulate division of the cambium (AVERY, BURKHOLDER, CREIGHTON 1937). When the buds are removed it is possible to stimulate the formation of secondary xylem by the application of a synthetic auxin, as SNOW (1935) discovered in sunflowers, and β-indole-acetic acid, as WAREING (1958) discovered in sycamore. With the use of gibberellic acid on sycamore with the buds removed, undifferentiated tissue was formed instead of xylem (WAREING 1958).

Methods and Results

1. For the experiment on cut-off shoots with the buds removed, shoots from the Velkopavlovická variety were used. They were cut from the tree on December 6, 1959 and cut into lengths each having 12 buds. Buds were left on five shoots and from ten others they were cut off with a razor blade. The bases of the shoots were immersed in dishes of water and placed in the laboratory at a temperature of 18—20° C. On December 9, the bud scars of five shoots were painted with a pure lanolin paste and the other five twigs with a paste of lanolin and β-indole-acetic acid (250 mg. β-indole-acetic acid dissolved in 4 ml. of 96% alcohol and made up with distilled water to 25 ml. This solution was mixed into 28 g. of lanolin). The buds on the shoots began to open on December 20. On December 28 an evaluation was carried out on hand cut preparations.

On the shoots where the buds remained, new xylem was formed on the side of the shoot beneath the breaking buds. This had a thickness in each internode of about three layers of trachea (cca 80 μ) whereas there was only one layer on the opposite side. Here and there on the opposite side to the bud, isolated trachea only were formed. In shoots with the buds removed and the sites painted with a paste of β-indole-acetic acid, a normal increase of xylem consisting of three layers of trachea (cca 80 μ) was formed round the circumference and for the whole length of the shoots. In no case did cambium form new tissue in shoots with buds removed and only lanolin applied. No pathological changes were found in cambium.

2. Experiments with the removal of buds from planted-out trees.

a) In the year 1959 on January 29, nine one-year-old graftlings of apricot (Sabinovská variety, plum stock, local variety) planted out in the Autumn of 1958 in pots, were brought into glass cabinets in the laboratory (temperature 18—20° C, humidity 50—80%). On January 30, all the buds were taken from six of the trees. The trees were examined daily during the experiment and any adventitious buds removed. On April 2 the experiment was evaluated. The control trees had sprouted and to that day had increased up to 15 cm. in length (the longest). The trees were cut up and the increase of new xylem measured on hand-cut microscopic sections at the base of the stem (above the grafting spot), in the fork of the branches (30 cm. above the base) and in the shoots. Table 1.

The results are shown in Tab. 1. From the table it is obvious that in trees where the buds remained, new xylem was formed from the shoots down to the base of the stem. One layer of trachea was formed in the shoots while at the base of the stem 2 (—3) layers. In trees with the buds removed, no new xylem formed in the shoots, but 1 (—2) layers of trachea were formed at the base of the stem.

Table 1. Formation of new xylem in one-year old apricot trees with the buds removed and in control trees (1959)

Tree		Base of stem		Stem at branch fork		Shoot	
		layers of trachea	μ	layers of trachea	μ	layers of trachea	μ
With buds	1	2—3	180	2	160	1	80
	2	2(—3)	170	2	160	1	75
	3	2	160	1	80	1	80
Without buds	1	1	80	1	80	0	0
	2	1	80	solitary	0—70	0	0
	3	1	70	1	70	0	0
	4	1—(2)	70(—160)	1	70	0	0
	5	2	170	1—(2)	170	0	0
	6	1	70	solitary	0—60	0	0

Table 2. Formation of new xylem in two-year old apricot trees (with the buds removed and control trees) (1960)

Tree		Base of stem		Stem at branch fork		Upper part of stem		Shoot	
		layers	μ	layers	μ	layers	μ	layers	μ
With buds	1	1	60	1	60	1	60	1	50
	2	1	50	2	150	1	60	1 (—2)	80
	3	1	70	1	40	1	60	1	50
	4	1	50	1	80	1	80	1	40
	5	1	60	2	130	2	170	1	40
Without buds	1	0	0	solitary	0—70	0	0	0	0
	2	1	80	1	60	0	0	0	0
	3	1	70	1	50	1	40	0	0
	4	solitary	0—70	1	60	0	0	0	0
	5	1	50	1	60	0	0	0	0

b) In the year 1960 the experiment was repeated with two-year old trees of the Sabinovská variety, plum stock. The trees were planted out in flowerpots in Autumn 1958 and left outside until December 22 1959, when they were brought into the glasshouse. On December 30 and 31 the buds on five trees were removed. During the experiment, adventitious buds which appeared were removed. Five trees were left as controls. The experiment was evaluated on February 1, 1960. The control trees had formed sprouts 10—15 cm. in length (the longest). The new xylem was measured on hand-cut microscopic sections at the base of the stem, in the fork of the branch (cca 70 cm. above the base), in the main shoot (stem continuation), and in the side shoots. Five shoots from each tree were studied.

The results given in Tab. 2 show that in these two-year old trees also with but one exception, new xylem, one layer of trachea thick was formed at the base of the stem even in trees with the buds removed. New xylem did not form in the shoots.

Table 3. Formation of xylem in one-year old apricot trees with buds removed and in control trees (1960)

Tree		Base of stem		Under branch fork		At branch fork		Shoot	
		layers	μ	layers	μ	layers	μ	layers	μ
With buds	1	2 (—3)	120 (—160)	3 (—4)	150 (—180)	—	—	3—4	100
	2	1	50	1	60	—	—	5	190
	3	1 (—2)	80	2	80	—	—	5	190
Without buds	1	2 (—3)	130	1	60	0	0	0	0
	2	1	70	solitary	0 (—60)	0—1	0—60	0	0
	3	solitary*	150**	0	0	0	0	0	0

* formation of one layer of resin cavities, solitary trachea over them

** together with resin cavities

Table 4. Formation of new xylem in three-year old apricot trees with buds and without buds (1960)

	Tree	Base of stem				Upper part of stem				Two year old branches				Shoot			
		6. 5.		13. 5.		6. 5.		13. 5.		6. 5.		13. 5.		6. 5.		13. 5.	
		layers	μ	layers	μ	layers	μ	layers	μ	layers	μ	layers	μ	layers	μ	layers	μ
With buds	1	1	100	2	250	1	100	2	180	—	—	2	160	1	80	3 (—4)	180—(250)
	2	1	120	2	240	1	100	1—2	160—(200)	—	—	2—3	150—190	1	60	3	200
	3	1	140	2—3	240—260	1	120	2	200	—	—	2	160	1	80	3	200
Without buds	1	1	60	1	60	1	60	1	60	—	—	0	0	0	0	0	0
	2	1	50	1	50	1	60	1	60	—	—	soli-tary	0—60	0	0	soli-tary	0—60
	3	1	60	1	60	1	50	1	50	—	—	0	0	0	0	0	0

c) The same experiment with the same conditions was carried out on one-year old trees planted out in Autumn 1959, Velkopopovická variety, seedling stock. The buds were removed on three trees on December 30, 1959, three were left as controls. The experiment was evaluated on March 10, 1960. By that time, the control trees had sprouts of 10 cm. (the longest). Samples from the base of the stem, from the stem beneath the branch fork and from the shoots were examined microscopically.

In this experiment too, new xylem one layer of trachea thick was formed at the base of the stem of budless trees as can be seen in Tab. 3.

d) An experiment with removed buds from trees planted out under natural conditions was also carried out. One-year-old trees of the Velkopopovická variety, plum stock, were planted out in the Spring of 1958. On February 24, 1960 all the buds on three trees were removed and three trees left as controls. During the experiment the adventitious buds which appeared were removed. On May 6 when the buds were breaking, samples were taken from the base of the stem, the top of the stem and from the shoots. On May 13, samples were taken from the same places and also from two-year old branches. Up till that day, the control trees had the longest sprouts — 15 cm.

In this experiment too, one layer of trachea formed during breaking (May 6) with a smaller diameter, however, than in the control trees. By May 13 there were already three layers of trachea in the control trees, while in the trees without buds, there was still only the one layer which had formed during the breaking of the buds in the control trees (Tab. 4).

3. A study of the formation of new xylem in different parts of the tree in connection with the developing of the buds.

a) On April 8, 1959 at the time of the breaking of the buds, samples were taken from the shoots 1 cm. below the bud and from the base of the stems of three trees and from two trees samples were taken from the stem, the base of the main shoot, a three-year old branch and from a shoot 1 cm. below a bud. The trees were seven-year-old, variety Velkopopovická, stock durancie (one tree was a red plum). Planting out at Holovousy.

b) On May 5, and 7, 1960, samples were taken from trees at the time of the breaking of the buds at Studenec near Jilemnice (from one five-year and two fourteen-year-old

Table 5. Formation of new xylem in 18 apricot trees, in various parts of the tree

Locality	Tree No.	Base of stem Layers of trachea	Upper part of stem Layers of trachea	3—5 year-old branch Layers of trachea	2 year-old branch Layers of trachea	Shoot Layers of trachea
	1	1	—	—	—	1
	2	solitary	solitary	—	—	1
a	3	0	resin cavities	—	—	solitary
	4	solitary	0	0	0	solitary
	5	1	1	solitary	0	solitary
	1	solitary	—	1	0	solitary*
b	2	solitary	—	0	0	solitary*
	3	solitary	—	solitary	0	solitary*
	1	1	—	—	—	solitary*
c	2	1—(2)	—	—	—	solitary*
	3	1	—	—	—	solitary*
	4	1	—	—	—	solitary*
	1	2	1	1	solitary	1
	2	2(—3)	parenchymatous	solitary	1	1—2
	3	2—(3)	1	0	1	1
d	4	2	1	solitary	1	1
	5	1	1	solitary	solitary	2
	6	1	1	1	solitary	1

* a continuous layer of trachea is formed only immediately beneath the bud

trees). The samples were taken from the base of the stem, from two-year-old branches and from a shoot, one cm. below a bud.

c) On May 5, 1960, the formation of new xylem in three apricot seedlings just at the breaking of the buds was studied. Two-year-old trees from the nursery at Vidochov near N. Paka were used. The base of the stem and shoots 1 cm. below a bud were examined microscopically.

d) On May 2, 1960 samples were taken from apricots with bursting buds (the first leaves were sprouting from the buds). They were eight-year-old trees from the plantations at Holovousy: Velkopopovická variety, durancie stock (three trees), St. Julien (two trees), Red plum (one tree).

The results are summarised in Tab. 5. It is apparent from the table that after the breaking of the buds there is always a more strongly developed layer of new xylem at the base of the stem than in the branches. New xylem always forms simultaneously at the base of the stem and in the shoots under the leaf buds when they are breaking. The formation of xylem in shoots before the buds break was not observed. The formation of xylem in the branches, especially 2—3 year old, and in the shoots farther from the breaking buds as well as on the side opposite the buds, lags behind the formation of xylem in the base of the stem.

On the basis of the previous experiments (with removed buds), the possibility can be allowed that new xylem begins to form at the base of the stem of apricots independently of the breaking buds.

Discussion

In the first experiment, the known fact was confirmed for apricot shoots, that β-indole-acetic acid stimulates the formation of new xylem when the buds are removed. In experiments with buds removed, the formation of new xylem in the stem of apricots occurred even in the absence of breaking buds. The explanation of this fact is that either growth substances are formed in the stem which stimulate the division of cambium even without the presence of buds or that the adventitious buds, that made their appearance chiefly on the branches of the stem, formed sufficient quantities of growth substances which stimulated cambium to form new xylem, in spite of the fact that they were removed in time, i.e. before breaking. The study of the trees at the time of the breaking of the buds showed that new xylem forms only under breaking leaf buds (according to my observations in the flowering season, new xylem does not form under the flower buds). I never observed the formation of xylem 1 cm. below the buds before their breaking. ADEFOGED (1952) says that new xylem forms several days before sprouting at the base of buds in all the trees he studied. He probably studied the formation of xylem right at the point of attachment of the bud.

In this work I have not studied whether new xylem is formed at the base of the stem before the breaking of the buds. When they broke, however, xylem was formed simultaneously under the buds and at the base of the stem. In 2—3 year old branches xylem was either not formed at all, or there were only solitary trachea. The foregoing observations are in contradiction with the prevailing conception according to which cambium first begins to form new xylem under the breaking buds and that this formation proceeds basipetally right to the stem. Some older works (BROWN 1915, KNUDSON 1916) also show that these conceptions are not commonly valid. As has been said, however, these works were considered erroneous.

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Образование вторичной ксилемы у абрикоса без участия распускающихся почек

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В предлагаемой работе автор исследовал образование вторичной ксилемы у абрикоса в зависимости от распускающихся почек. В отрезанных годичных приростах, у которых были удалены почки, вторичная ксилема образовывалась лишь после применения β-индолилуксусной кислоты. У 1—3-летних деревцев с удаленными почками имело место образование ксилемы толщиной в 1—2 слоя трахей только в стволе, а не в годичных приростах или в 2-летних ветках. При изучении хода развития ксилемы у деревьев абрикоса разного возраста было установлено, что новая ксилема образуется при распускании листовых почек одновременно в годичном приросте под почкой и у основания ствола. Образование ксилемы запаздывает в 2 –3-летних ветвях и в годичном приросте на стороне противоположной почке. Эти наблюдения противоречат до сих пор приводимой схеме, согласно которой у деревьев образование ксилемы имеет место сначала под распускающейся почкой и затем постепенно распространяется в базальном направлении до самого основания ствола.

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Application of Some Inhibitors in Studying the Physiology of Vernalization

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Použití některých inhibitorů při studiu fysiologie jarovisace

1. V průběhu jarovisace ozimé pšenice Hodonínské holice byla aplikována řada inhibitorů: kyanid draselný, α,α'-dipyridyl, 2,4-dinitrofenol, kys.monojodoctová, fluorid sodný a kumarin. U takto jarovisovaného materiálu byla zjišťována různá kriteria rychlosti vývoje, jako délka období do metání a procento generativních rostlin. Bylo zjištěno, že všechny uvedené látky inhibovaly jarovisaci. Kyanid draselný, α, α'-dipyridyl a 2,4-dinitrofenol jarovisační vliv nízkých teplot zcela potlačily. Specifičnost tohoto efektu byla sledována srovnáním s vlivem inhibitorů na jarní odrůdu (Niva), která je bez požadavku jarovisace. Pouze v případě fluoridu sodného byla ovlivněna rychlost vývoje jarní odrůdy.

2. Výsledky pokusů tak ukázaly na značně specifické ovlivnění jarovisace danými inhibitory. Zdá se, že jarovisace je úzce spojena s oxydativní fosforylací. Z tohoto hlediska by byla potvrzena i dřívější zjištění o závislosti jarovisace na aerobních podmínkách.

Summary

1. In the course of vernalization of winter wheat Hodonínská holice a number of inhibitors were used: potassium cyanide, α,α'-dipyridyl, 2,4-dinitrophenol, iodoacetic acid, sodium fluoride and coumarin. On comparing the effect with that on a spring wheat without vernalization requirement it was shown that all the above compounds, with the exception of sodium fluoride, acted as quite specific inhibitors of vernalization. Potassium cyanide, α,α'-dipyridyl and 2,4-dinitrophenol blocked vernalization completely.

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2. The experimental results are in agreement with the data on the dependence of vernalization on aerobic conditions and pointed to a possible connection with oxidative phosphorylation. The data obtained did not provide sufficient basis for a concrete hypothesis to be established about the mechanism of vernalization.

Introduction

In studying the mechanism of a number of physiological processes plant physiology has frequently made use of specific metabolic inhibitors. In the field of flowering induction, however, apparently on account of the complexity of this phenomenon, but a few papers were published and most of these deal with photoperiodic phenomena. In investigation of vernalization this approach was used only exceptionally. CHOUARD and POIGNANT (1951), in a pilot experiment with winter wheat, examined a number of respiratory inhibitors and antiauxins. CHAKRAVARTI and PILLAI (1955), and CHARKAVARTI (1958) inhibited the vernalization process with triiodobenzoic acid and maleic hydrazide. GÜNTHER (1960) with potassium cyanide. The work of KOJIMA and co-workers (1957) follows similar lines. In the work described here this approach was used for studying the physiology of vernalization. The present paper contains the results of comparative experiments dealing with the specificity of individual inhibitors in influencing vernalization of winter cereals.

In the experiments described here a number of inhibitors, including inhibitors of the terminal oxidases, glycolysis, the Krebs cycle, phosphorylation processes and of antiauxin-type inhibitors, were used. An attempt was made to establish the differences between the effect of individual groups of inhibitors investigated. The paper includes experiments with inhibitors which produced unequivocal results on repetition.

Material and Methods

Winter wheat *Triticum aestivum*, var. *miltarum* Al. of the sort Hodonínská holice, and spring wheat *Triticum aestivum* var. *erythrospermum* KORN. of the sort Niva, were used in the experiments. The Hodonínská holice belongs to sorts with a very marked vernalization requirement and on late spring sowing most plants, without vernalization, remain vegetative. The vernalization period for seeds containing 52—55% water is 55 ± 5 days (KREKULE 1960). Grains representing the offspring of a single plant were used for the experiments.

The inhibitors were applied in two ways. In the first method, grains were soaked in aqueous solutions of inhibitors for 12 hr. at 25° C, then left for another 12 hr. at the same temperature in an atmosphere of 100% relative humidity and finally transferred to vernalization temperatures. Vernalization was carried out at 2 ± 1° C for 35 days in 100 ml. Erlenmayer flasks closed with a wet cotton-wool plug. In each variant a total of 250—300 grains were vernalized. After vernalization, the grains were washed for 10 hr. in running tap water. The degree of sprouting was determined in the individual variants and the grains which were in no way damaged were sown in a field experiment, together with non vernalized controls. The second method of application of inhibitors consisted in a different vernalization technique. All pre-vernalization manipulations were carried out in the same way as in the first case. The grains

were vernalized in Petri dishes moistened with the corresponding inhibitor solution (8 ml./100 g. glassgrinder's sand). Grains vernalized in distilled water were used in both cases. Vernalization temperatures and the treatment after vernalization were the same as in the first case. The scheme of treatment and the evaluation of the experiment was the same in both variants of the application of inhibitors.

The character of the results is analogous in both cases in that the degree of inhibition was lower during vernalization in moistened sand in the presence of greater ecological and morphological variability of the material. The Tables show only the data obtained by the first method of vernalization. The data on growth during vernalization have not been treated statistically and in the description they are considered merely as observations. The control variants (vernalized with distilled water) were in the following stage of germination at the termination of vernalization: Hodonínská holice, coleoptile 3 mm., first root 5—6 mm., second root 3—6 mm.; Niva, coleoptile 2—3 mm., a single root 2—4 mm.

Each variant was sown out in two rows (75 grains each) 10 cm. apart. The evaluation was carried out with reference to the duration of the phase "sowing-beginning of earing" (10% plants earing) and the phase "sowing-earing" (more then 50% plants earing). After the end of the experiment, the number of plants in the vegetative and generative stage was determined in each variant (plants displaying at least differentiation of flower primordia are considered to be in the generative stage). The entire experiment was sown out on two different dates: May 2, 1959, and May 22, 1959, i.e. at a time when the winter sort without vernalization remains for the most part in the vegetative state. The character of the results at both dates of sowing was approximately the same but the degree of inhibition was higher on the second date (higher temperatures impair vernalization conditions).

The results obtained on the later date are not included in the Tables.

Glass-distilled water was used for the preparation of all solutions and for all manipulations, unless otherwise stated. The inhibitor concentrations used were determined on the basis of data in the literature as well as after a preliminary experiment where the effect of the given inhibitor on growth of over-ground parts and roots of the sort Hodonínská holice was examined. Concentrations were then used which inhibited growth markedly and which had practically no effect using the given criteria.

Results

Table 1 summarizes results obtained with potassium cyanide. At the highest concentration a complete suppression of the vernalization influence of low temperatures was achieved with the winter sort and even at 5×10^{-4}M cyanide inhibited vernalization quite distinctly (the proportion of plants in the generative stage was already rather high). The spring sort was not affected at all by the above concentrations. Germination was inhibited in both wheat sorts only by the highest concentration, the lowest concentration, on the contrary, somewhat enhancing the development. Table 2 shows the data obtained with α,α'-dipyridyl. The highest concentration of this inhibitor blocked vernalization completely. The concentration lower by one order of magnitude displayed but a minute retarding effect on the development (not influencing the proportion of generative plants). The growth rate of the spring sort was not at all affected by α,α'-dipyridyl. Germination was inhibited in both sorts by 10^{-3}M inhibitor in the course of vernalization.

Table 3 summarizes the data obtained on applying 2,4-dinitrophenol. The highest concentration blocked vernalization completely and the plants then behaved like the non-vernalized controls. A concentration lower by one order of magnitude still affected vernalization quite distinctly which was displayed not only in a longer period before taking to ear but also in a decreased per-

Table 1 (potassium cyanide)

Variant	Sowing-beginning of earing (days)	Diff. (days)	Sowing-earing (days)	Diff. (days)	Number of plants	Per cent generative phase
Hh — 10^{-3}M	117	+ 56	*	—	40	22·5
Hh — 5×10^{-4}M	84	+ 23	*	—	42	74
Hh — 10^{-4}M	59	— 2	66	— 14	67	95·5
Hh — CV	61	—	80	—	61	85·5
Hh — C	85	+ 24	*	—	77	14·5
N — 10^{-3}M	51	—	53	—	99	100
N — 5×10^{-4}M	51	—	53	—	122	100
N — 10^{-4}M	51	—	53	—	98	100
N — CV	51	—	53	—	102	100
N — C	53	+ 2	54	+ 1	95	100

Legend: Hh — Hodonínská holice (winter sort), N — Niva (spring sort); CV — vernalized control, C — non-vernalized control. Differences in the onset of a given phenological phase are related to the vernalized control of the given sort.* The variant did not attain the given phenological phase.

Table 2 (α,α'-dipyridyl)

Variant	Sowing-beginning of earing (days)	Diff. (days)	Sowing-earing (days)	Diff. (days)	Number of plants	Per cent generative phase
Hh — 10^{-3}M	*	—	*	—	42	26
Hh — 10^{-4}M	69	+ 8	81	+ 1	67	84
Hh — CV	61	—	80	—	61	85·5
Hh — C	85	+ 24	*	—	77	14·5
N — 10^{-3}M	52	+ 1	54	+ 1	102	100
N — 10^{-4}M	52	+ 1	54	+ 1	106	100
N — CV	51	—	53	—	102	100
N — C	53	+ 2	54	+ 1	95	100

Legend: as in Table 1

centage of generative plants. The lowest concentration used did not affect either the period before taking to ear or the percentage of generative plants. Inhibition of germination was exhibited by both sorts in a low degree only with the highest concentration. The spring sort was not affected by the application of 2,4-dinitrophenol.

Table 4 contains data of the effect of iodoacetic acid. The highest concentration shown (at 10^{-2}M-iodoacetic acid the grains did not germinate) partly slowed down the process of vernalization, while the percentage of generative plants was decreased only very little. At 5×10^{-4}M, the course of vernalization was practically unaffected. In the spring sort the rate of development was not influenced at all. None of the variants exhibited inhibition of germination.

Table 3 (2,4-dinitrophenol)

Variant	Sowing-beginning of earing (days)	Diff. (days)	Sowing-earing (days)	Diff. (days)	Number of plants	Per cent generative phase
Hh — 10^{-3}M	*	—	*	—	49	22
Hh — 10^{-4}M	76	+ 15	85	+ 5	49	55
Hh — 10^{-5}M	69	+ 8	76	— 4	36	89
Hh — CV	61	—	80	—	61	85·5
Hh — C	85	+ 24	*	—	77	14·5
N — 10^{-3}M	52	+ 1	54	+ 1	91	100
N — 10^{-4}M	52	+ 1	54	+ 1	104	100
N — 10^{-5}M	51	—	53	—	93	100
N — CV	51	—	53	—	102	100
N — C	53	+ 2	54	+ 1	95	100

Legend as in Table 1

Table 4 (iodoacetic acid)

Variant	Sowing-beginning of earing (days)	Diff. (days)	Sowing-earing (days)	Diff. (days)	Number of plants	Per cent generative phase
Hh — 10^{-3}M	83	+ 22	87	+ 7	65	83
Hh — 5×10^{-4}M	69	+ 8	83	+ 3	63	93·5
Hh — CV	61	—	80	—	61	85·5
Hh — C	85	+ 24	*	—	77	14·5
N — 10^{-3}M	51	—	53	—	110	100
N — CV	51	—	53	—	102	100
N — C	53	+ 2	54	+ 1	95	100

Legend as in Table 1

Table 5 (sodium fluoride)

Variant	Sowing-beginning of earing (days)	Diff. (days)	Sowing-earing (days)	Diff. (days)	Number of plants	Per cent generative phase
Hh — 10^{-2}M	84	+ 23	103	+ 23	66	86
Hh — 10^{-3}M	73	+ 12	92	+ 12	60	90
Hh — 10^{-4}M	69	+ 8	73	— 7	83	98
Hh — CV	61	—	80	—	61	85·5
Hh — C	85	+ 24	*	—	77	14·5
N — 10^{-2}M	76	+ 25	79	+ 26	75	80
N — 10^{-3}M	51	—	53	—	103	100
N — 10^{-4}M	51	—	53	—	102	100
N — CV	51	—	53	—	102	100
N — C	53	+ 2	54	+ 1	95	100

Legend as in Table 1

Table 5 shows the results of inhibition with sodium fluoride. At the highest concentration, both the winter and the spring sort were affected to the same degree and this effect appears to be quite non-specific. At 10^{-3}M, the rate of vernalization was decreased (without affecting the percentage of plants in the generative phase of development); only the highest concentration was observed to inhibit germination in both sorts.

Table 6 (coumarin)

Variant	Sowing-beginning of earing (days)	Diff. (days)	Sowing-earing (days)	Diff. (days)	Number of plants	Per cent generative phase
Hh — 10^{-3}M	85	+ 24	104	+ 24	39	82
Hh — 10^{-4}M	81	+ 20	98	+ 18	49	82
Hh — CV	61	—	80	—	61	85·5
Hh — C	85	+ 24	*	—	77	14·5
N — 10^{-3}M	52	+ 1	53	—	86	100
N — 10^{-4}M	52	+ 1	53	—	86	100
N — CV	51	—	53	—	102	100
N — C	53	+ 2	54	+ 1	95	100

Legend as in Table 1

Table 6 presents results obtained with coumarin. At both concentrations used the same degree of inhibition was obtained, manifesting itself only in a prolongation of the period to earing. The development of the spring sort was not affected by coumarin. At 10^{-3}M, germination was completely suppressed in both sorts.

Discussion

A number of difficulties are encountered with the use of inhibitors in studying vernalization. Hitherto only an ecological characterization of vernalization has been made while our concepts about its physiological mechanism are very limited and fragmentary. Thus it is not feasible to decide on the use of a certain inhibitor and to predict its effect. For this reason it is possible to define the specificity of action of individual inhibitors on vernalization only on the basis of their differential effect, i.e. by comparison with their effect on a wheat sort which has no vernalization requirements (the Niva sort used here). A serious problem is represented by the question of long-time application of inhibitors and maintenance of a given degree of inhibition which has not hitherto been satisfactorily solved, either from the methodical or from the theoretical point of view. There is not only the problem of a suitable mode of application with respect to the binding of the given inhibitor (affinity of the inhibitor, possibility of its metabolizing etc.) but also the fact that the reaction of inhibited tissue can change due to ontogenetic age or to some adaptation reaction. In this experiment, the effect is interpreted according to

the final symptom produced. The results obtained, however, were in a number of cases so unequivocal that they can only be used as orientation indicators.

In the experiments described here, potassium cyanide inhibited vernalization completely. Cyanide was used for inhibition of vernalization by CHOUARD and POIGNANT (1951) whose results (presented only as a relative value) point basically to a strong inhibitory influence of this compound. Similar results obtained also GÜNTHER (1960.) If it is assumed that this inhibitor blocks the cytochrome-catalyzed electron transfer to atmospheric oxygen, the results can but confirm the previous findings of a number of authors on the indispensability of aerobic conditions for vernalization (GREGORY and PURVIS 1938, FILIPPENKO 1940, SECHET 1953, AGINYAN 1958, GÜNTHER 1960). This view is further supported by the fact that in the wheat seedlings used here (the results are not presented) active cytochrome oxidase inhibited by cyanide was detected. The fact that the lowest concentration of cyanide (10^{-4}M) had the opposite effect on accelerating growth cannot be explained on the basis of these results. It may depend on the increase in respiratory activity produced by these low inhibitor concentrations, which was also observed in our experiments. These data may be to some extent correlated with the result obtained with α,α'-dipyridyl. At 10^{-3}M, vernalization was completely blocked (germination was inhibited non-specifically). If the assumed point of action of α,α'-dipyridyl is aconitase (JAMES 1956) acting "in series" with terminal oxidases we may be dealing with a similar type of effect as with cyanide.

I feel that, with respect to vernalization, the inhibition of oxidative phosphorylation connected with the activity of terminal oxidases is of great importance. The complete inhibition of vernalization by 2,4-dinitrophenol can perhaps be explained in this sense directly by the blocking of oxidative phosphorylation. Where the formation of high-energy phosphate bonds as the source energy for vernalization is stopped, the inhibition is highest (with 2,4-dinitrophenol, cyanide and α,α'-dipyridyl). This view, however, requires further experimental verification but for the time being it suffices for the explanation of identical effects obtained with the three above described inhibitors (azide had the same effect as cyanide) and it suggests the possible link with the prerequisite of aerobic conditions, commonly shown as one of the physiological characteristics of vernalization.

On applying iodoacetic acid and fluoride the effect of vernalization is but partly reduced, the effect of fluoride being quite nonspecific. In experiments of CHOUARD and POIGNANT (1951) vernalization was almost completely blocked by these agents. Both inhibitors block glycolysis (iodoacetic acid quite non-specifically). The above results concerning the rather insignificant degree of inhibition do not permit of any conclusions to be made with regard to the possible relationship between the glycolytic pathway and vernalization. Coumarin produced only partial inhibition of vernalization (the percentage of generative plants was not affected, however). On account of the fact that the mechanism of action of this compound is not yet satisfactorily known (LEOPOLD 1955) and that it is not possible in this case to explain the inhibition by the mere antagonism with growth factors (germination was inhibited in the spring sort, as well) the question of the effect on vernalization is not yet clear.

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Использование некоторых ингибиторов при изучении физиологии яровизации

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1. В течение яровизации озимой пшеницы сорта Годишиска голице был применен ряд ингибиторов: цианистый калий, α, α′-дипиридил, 2,4-динитрофенол, монойодоуксусная кислота, фтористый натрий и кумарин. У яровизованного таким образом материала определялись различные критерии скорости развития, такие как длина периода до колошения и процент растений в генеративной фазе развития. Было установлено, что все приведенные выше вещества ингибируют яровизацию. Цианистый калий, α, α′ дипиридил, а также 2,4 динитрофенол совершенно подавили яровизационное влияние низких температур. Специфичность этого эффекта изучалась путем сравнения с воздействием ингибиторов на яровой сорт (Нива), который не требует яровизации. Лишь в случае фтористого натрия наблюдалось воздействие на скорость развития ярового сорта.

2. Результаты опытов свидетельствуют о весьма специфическом влиянии данных ингибиторов на яровизацию. Кажется, что процессы яровизации тесно связаны с окислительной фосфорилляцией. С этой точки зрения была бы подтверждена уже ранее установленная зависимость яровизации от аэробных условий.

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The Size of Guard Cells of Stomata of a Natural Polyploid Series of the Genus *Triticum* L.

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Velikost svěracích buněk průduchů u přirozené polyploidní řady rodu *Triticum* L.

1. Byla studována délka svěracích buněk průduchů a počet průduchů na plošnou jednotku u 20 odrůd deseti různých druhů polyploidní řady rodu *Triticum* L. na otiscích nejvyššího listu, zhotovených acetonovým lakem.

2. Délka průduchů stoupá se vzrůstajícím počtem chromosomů (diploidní *T. monococcum* L. 33,4 μ, tetraploidní druhy mimo *T. timopheevi* Zhuk. 42,0 μ, hexaploidní druhy 45,4 μ). Odlišnou velikost průduchů má *T. timopheevi* Zhuk. (48,1 μ). Je to další důkaz oddělenosti tohoto druhu od ostatních tetraploidních pšenic. Uvnitř jednotlivých skupin o stejném počtu chromosomů mají větší délku svěracích buněk druhy fylogeneticky pokročilejší. To je snad možno spojovat s celkovou mesofylisací rodu v průběhu fylogenese. Nelze ovšem zanedbávat ekologické vlivy.

3. Podle působení na délku průduchů u jednotlivých skupin lze jednotlivé genomy seřadit do této sestupné řady: A > G > B > D.

4. Počet průduchů na plošnou jednotku nejeví žádnou souvislost s počtem chromosomů. Na počet průduchů mají zřejmě vliv více ekologické faktory než vnitřní vztahy genetické.

Summary

1. The length of guard cells of stomata and the number of stomata per unit area were studied in 20 varieties of 10 different species of a polyploid series of the genus *Triticum* L., using replicas of the topmost leaf prepared with acetone varnish.

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2. The length of stomata increases with increasing number of chromosomes (diploid *T. monococcum* L. 33·4 μ, tetraploid species with the exception of *T. timopheevi* Zhuk. 42·0 μ, hexaploid species 45·4 μ). *T. timopheevi* has a different size of stomata (48·1 μ). This serves as further evidence for the separate character of this species as compared with the other tetraploid species of wheat. Within the individual groups possessing the same number of chromosomes the phylogenetically more developed species have longer guard cells. This might be associated with the general mesophylization of the genus in the course of phylogeny. Ecological influences must not be disregarded, however.

3. According to the effect on the length of stomata in the individual groups the genomes can be arranged in the following descending series: A > G > > B > D.

4. The number of stomata per unit area does not appear to be related to the number of chromosomes. It is apparently affected more by ecological factors than by intrinsic genetic relationships.

Introduction

Species of the genus *Triticum* L. form a natural polyploid series containing diploid, tetraploid and hexaploid species (Sakamura 1918). Octoploid and decaploid amphidiploid interspecies hybrids also exist (Zhebrak 1957). It is known that an increase in chromosome number is manifested by an enlargement of cells and cell nuclei as well as by a general more robust habit of the plant. These changes are best marked in specialized cells such as pollen grains or stomatal guard cells (Stebbins 1957).

In the genus *Triticum* L. a proportionality between the pollen grain size and the number of chromosomes has been established by Firbas (1937). The problem was taken up in more detail by Cetl (1960). The increase in size of the stomatal guard cells with increasing chromosome number was also demonstrated in camomile (Chládek, Košová and Hrubý 1958).

In the genus *Triticum* L. this relationship has not been studied so far. The present paper deals with the investigation of the size of stomata and of their number per unit area.

Material and Methods

The size of the guard cells of stomata and the number of stomata per unit area was examined in 20 samples of 10 different species of the genus *Triticum* L. The diploid group was represented by *T. monococcum* L. var. *hornemanii* Clem. In the tetraploid group there were *T. timopheevi* Zhuk. var. *timopheevi*; *T. dicoccum* Schrank var. *farrum* Korn.; *T. durum* Desf. var. *hordeiforme* Korn. (Hordeiforme 10); *T. durum* Desf. var. *candicans* Meist. (Candicans 76 10); *T. durum* Desf. var. *melanopus* Alef. (Melanopus 39 and Melanopus 69); *T. durum* Desf. var *leucurum* (Leucurum 33); *T. turgidum* L. var. *salomonis* Korn; *T. polonicum* L.; *T. persicum* Vav. var. *stramineum* Zhuk. and *T. persicum* Vav. var. *fuliginosum* Zhuk. The hexaploid

species were represented by: *T. vulgare* Vill. var. *caesium* Alef. (Caesium 111); *T. vulgare* Vill. var. *erythrospermum* Körn. (Niva); *T. vulgare* Vill. var. *milturum* Alef. (Milturum 321 and Podbořanka); *T. vulgare* Vill. var. *lutescens* Alef. (Lutescens 62 and Ratbořská); *T. compactum* Host. var. *erinaceum* Körn and *T. spelta* L. var. *duhamelianum* Körn.

All the forms studied were spring wheats and originated from the collection of cereals of the Department of Plant Physiology and Genetics of the Faculty of Natural Science of the Purkyně University in Brno.

Measurements were carried out on leaf replicas prepared by means of acetone varnish (Slavík 1954) from the lower third of the upper side of the topmost leaf at the time of taking to ear in June 1956. A micrometric eyepiece with an accuracy to 2·47 μ was used. In each sample a total of 300 stomata were measured in three leaves and their average length ($\bar{x}$), standard deviation (s), standard error ($s_{\bar{x}}$) and variation coefficient (v) calculated (Hrubý and Konvička 1954).

Results

The results of measurements are compiled in Table 1 and 2.

Table 1. Size of guard cells of stomata of *Triticum* L. species

Species (variety)	$\bar{x} \pm 3 . s_{\bar{x}}$	$\pm$ s	v (%)
2n = 14			
T. monococcum L.	33·4 ± 3 . 0·15	2·83	8·5
2n = 28			
T. timopheevi Zhuk.	48·1 ± 3 . 0·20	3·34	7·2
T. dicoccum Schrank.	41·2 ± 3 . 0·30	5·26	12·8
T. durum Desf. (Candicans 76/10)	42·9 ± 3 . 0·20	3·66	8·5
T. durum Desf. (Hordeiforme 10)	40·2 ± 3 . 0·21	3·75	9·3
T. durum Desf. (Melanopus 37)	41·5 ± 3 . 0·22	4·03	9·6
T. durum Desf. (Melanopus 69)	42·5 ± 3 . 0·24	4·04	9·5
T. durum Desf. (Leucurum 33)	43·6 ± 3 . 0·30	5·29	12·1
T. turgidum L.	45·6 ± 3 . 0·29	4·98	10·9
T. polonicum L.	42·8 ± 3 . 0·29	5·01	11·7
T. persicum Vav. var. *stramineum*	40·0 ± 3 . 0·21	3·55	8·9
T. persicum Vav. var. *fuliginosum*	39·8 ± 3 .0·24	4·18	10·0
2n = 42			
T. vulgare Vill. (Caesium 111)	45·5 ± 3 . 0·22	3·82	8·4
T. vulgare Vill. (Niva)	47·4 ± 3 . 0·25	4·22	8·9
T. vulgare Vill. (Milturum 321)	45·9 ± 3 . 0·23	3·91	8·5
T. vulgare Vill. (Podbořanka)	42·8 ± 3 . 0·23	4·13	9·7
T. vulgare Vill. (Lutescens 62)	43·0 ± 3 . 0·22	3·68	8·6
T. vulgare Vill. (Ratbořská)	47·9 ± 3 . 0·22	3·86	7·9
T. compactum Host.	43·2 ± 3 . 0·25	4·31	9·4
T. spelta L.	47·8 ± 3 . 0·29	4·93	10·3

Table 2. Number of stomata per mm.² in different species of *Triticum* L.

Species (variety)	$\bar{x} \pm 3 . s_{\bar{x}}$	s	v (%)
2n = 14			
T. monococcum L.	97·5 ± 3 . 0·38	10·68	11·0
2n = 28			
T. timopheevi Zhuk.	71·7 ± 3 . 1·13	8·84	12·3
T. dicoccum Schrank.	70·3 ± 3 . 1·77	13·74	19·5
T. durum Desf. (Candicans 76/10)	68·1 ± 3 . 1·79	13·79	20·3
T. durum Desf. (Hordeiforme 10)	100·5 ± 3 . 2·12	16·31	16·2
T. durum Desf. (Melanopus 37)	78·0 ± 3 . 1·50	11·63	14·9
T. durum Desf. (Melanopus 69)	87·0 ± 3 . 1·48	11·28	13·3
T. durum Desf. (Leucurum 33)	97·4 ± 3 . 2·33	17·98	18·5
T. turgidum L.	71·2 ± 3 . 1·86	14·33	20·1
T. polonicum L.	75·0 ± 3 . 2·52	19·50	26·0
T. persicum Vav. var. *s ramineum*	84·3 ± 3 . 1·72	13·32	15·8
T. persicum Vav. var. *fuliginosum*	95·2 ± 3 . 2·17	16·83	17·7
2n = 42			
T. vulgare Vill. (Caesium 111)	74·9 ± 3 . 1·69	13·08	17·5
T. vulgare Vill. (Niva)	68·1 ± 3 . 2·75	12·23	18·0
T. vulgare Vill. (Milturum 321)	75·6 ± 3 . 1·25	99·64	12·8
T. vulgare Vill. (Podbořanka)	81·2 ± 3 . 2·09	16·12	19·9
T. vulgare Vill. (Lutescens 62)	109·0 ± 3 . 2·43	18·84	17·3
T. vulgare Vill. (Ratbořská)	81·4 ± 3 . 2·85	22·09	26·1
T. compactum Host.	93·5 ± 3 . 2·20	17·01	18·2
T. spelta L.	71·2 ± 3 . 1·67	13·00	18·2

Discussion

The results show that in the genus *Triticum* the length of stomata rises with increasing number of chromosomes. In the diploid *T. monococcum* L. it amounts to 33·4 μ, in the tetraploid species with the exception of *T. timopheevi* Zhuk. it is on the average 42·0 μ and in the hexaploid species it reaches 45·4 μ. A significantly different size of pores may be found with *T. timopheevi* Zhuk. (48·1 μ).

From the genetic point of view different genomes in somatic cells come into play with the individual wheat groups as suggested by Gaines and Aase (1926): AA in diploid cells, AABB in tetraploid cells and AABBDD in hexaploid cells. *T. timopheevi* Zhuk. has a different genome AAGG. As a relationship was suggested to exist between the genome composition and pollen grain size (Cetl 1960) it was attempted here to demonstrate a similar relationship with the size of stomata. If the length of stomatal guard cells is set equal to 100 then the size of these cells in the tetraploid species — with the exception of *T. timopheevi* Zhuk. — is equal to 126, in the hexaploid ones to 136 and in *T. timopheevi* Zhuk. as much as 144. The different position of *T. timopheevi* Zhuk. from the point of view of phylogeny is mentioned by

Sinskaya (1955) and emphasized by the fact that it produces sterile offspring with all other tetraploid wheats (Menabde 1948). Cetl (1960) ascribes the lowest value for the size of pollen grains of all tetraploid wheats to *T. timopheevi* Zhuk. If the influence of the individual genomes on the size of stomata is compared they may be arranged in the following descending series: A > > G > B > D. Thus the size of stomata displays an opposite relationship.

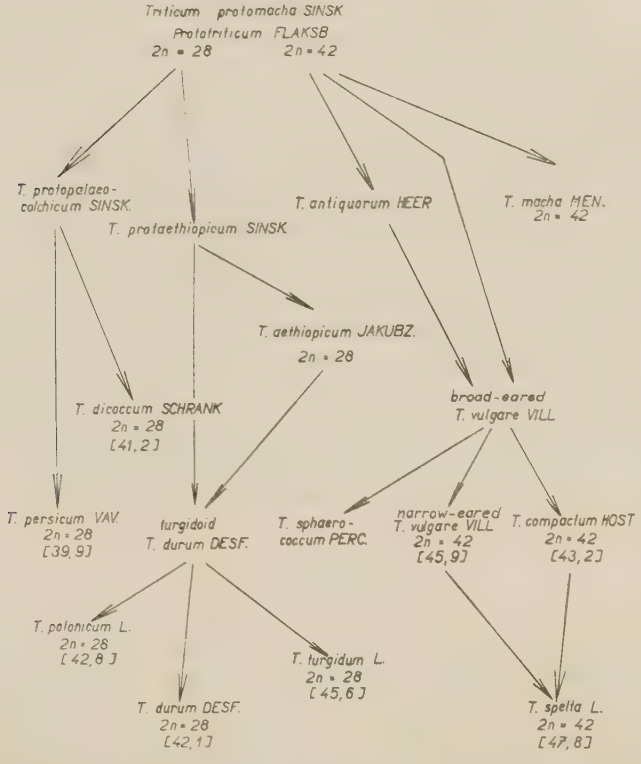

Fig. 1. Scheme of phylogenetic development of species of the genus *Triticum* L. (Simplified after Sinskaya). Figures in parentheses represent the average lengths of the guard cells.

Cetl (1960) found another deviation in the size of pollen grains of *T. turgidum* L. which has larger pollen grains than any other tetraploid wheat. The same deviation was observed in the size of stomata: *T. turgidum* L. has 45·6 μ while the average of the whole group is 42·0 μ. This may be explained in the same way as Cetl (1960) employs for the analogies in pollen grain size.

If the results of measurement of stoma length are compared with the phylogeny of wheat (Fig. 1) it may be seen that within the individual groups those species possess longer stomata which have undergone a longer phylogenetic development, i.e. those which are at the ends of the branches of the

developmental tree. The attached scheme due to SINSKAYA shows this relationship very markedly. For $2n = 28$, the turgidoid form of *T. durum* DESF. gave rise to *T. polonicum* L., *T. durum* DESF. proper and *T. turgidum* L. The present *T. durum* DESF. is obviously closest to the turgidoid *T. durum* DESF. which indicates that it has undergone relatively the shortest development and it really has the shortest stomata of all the three species. This relationship is even more pronounced in the hexaploid species *T. compactum* HOST. and *T. spelta* L. It follows from the above-mentioned scheme that *T. spelta* is most advanced in its development with correspondingh the longest stomata. On the other hand, however, it must be borne in mind that these relationships can be and in fact are influenced by a great many ecological factors. The increasing length of stomata may be interpreted in the more advanced species as a consequence of a mesophylization of the genus as described by SINSKAYA (1955). It appears that the intrinsic relationships are more reflected in the size of pollen grains as a reproductive organ. Nevertheless, even in the guard cells of the stomata the relationships are quite clearly displayed.

In further experiments the number of stomata per unit area was determined. The averages of individual groups are 97·5 for diploid wheats, 82·7 for tetraploid wheats, 83·8 for hexaploid wheats and 71·7 for *T. timopheevi* ZHUK., per mm.^{2}. It follows from these relationships that the number of stomata per unit area is not directly linked with the number of chromosomes as shown also by the considerable variability within the individual groups. This supports the assumption that the density of stomata is much more affected by ecological factors than by genetic or phylogenetic influences.

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Величина запирающих клеток дыхалец у естественного-полиплоидного ряда рода *Triticum* L.

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1. Измерялась длина запирающих клеток дыхалец и количество дыхалец на единицу площади у 20 сортов десяти разных видов полиплоидного ряда рода *Triticum* L. Измерения проводились на оттисках с самого верхнего листа, изготавливаемых при помощи ацетонового лака.

2. Длина дыхалец увеличивается с возрастанием количества хромозом (диплоидная *T. monococcum* L. 33,4 μ; тетраплоидные виды, кроме *T. timopheevi* Zhuk, 42,0 μ; гексаплоидные виды 45,4 μ). Величина дыхалец отличается лишь у *T. timopheevi* Zhuk. (48,1 μ). Последнее является дальнейшим доказательством обособления этого вида от остальных тетраплоидных видов пшеницы. Внутри отдельных групп с одинаковым числом хромозом виды, филогенетически более развитые, отличаются большими запирающими клетками дыхалец. Вероятно, это можно привести в связь с общей морфологией этого рода в течение филогенеза. Однако, нельзя не принимать во внимание и воздействие экологических факторов.

3. По влиянию на длину дыхалец у отдельных групп можно построить из отдельных геномов следующий нисходящий ряд: A > G > B > D.

4. Количество дыхалец на единицу площади не зависит от количества хромозом. На число дыхалец явно оказывают бóльшее влияние экологические факторы, чем внутренние, генетические отношения.

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L'action synergique de la Simazine et des viroses de plantes en tant que méthode de la détection éventuelle des maladies à virus

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Synergické působení Simazinu a virového onemocnění rostlin jako metoda pro možnost detekce virového ochuravění

Při studiu účinku Simazinu na virově nemocné rostliny bylo zjištěno, že dávky zálivkového roztoku Simazinu (0,33 mg nebo 0,50 mg na jednu rostlinu), které nemají žádný škodlivý účinek na zdravé rostliny, usmrcují rostliny virově nemocné. Tento synergismus účinku Simazinu a virového onemocnění byl pozorován u rostlin tabáku onemocnělých VTM, u ptačinců onemocnělých žloutenkou blízkou aster yellows, na rostlinách bramboru a předběžně na rostlinách chmelu.

Résumé

On a constaté, en étudiant l'effet de la Simazine sur les plantes atteintes de viroses, que les doses de la solution d'arrosage de la Simazine (0,33 mg ou 0,50 mg par une plante), n'ayant aucun effet nuisible sur les plantes saines, causent un dépérissement aux plantes malades. Cette synergie dans l'effet de la Simazine et des viroses attaquant les plantes a été observée sur les plantes du tabac infectées du VTM, sur l'alsine atteinte de la jaunisse, proche de l'aster yellows, sur les plantes de pommes de terre et au préalable sur les plantes du houblon.

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Introduction

On connaît relativement peu de l'action de la Simazine (Gast et al. 1956) 2 chlor 4,6 bis(ethylamino) triazine sur les plantes. Par exemple, il y a trois auteurs qui s'occupent dans leurs oeuvres à l'étude du mécanisme de l'effet de cette substance herbicide très active. Roth (1958) a constaté que la Simazine inhibe la photosynthèse chez la plante aquatique *Elodea* et augmente encore l'utilisation de l'oxygène lors de la respiration. Les systèmes déshydrasiques restent actifs. Gast (1958) a démontré par ses expériences sur les plantes *Coleus Blumei* et *Tropaeolum*, que l'inhibition de la photosynthèse résulte en l'arrêt da la biosynthèse de l'amidon. Cet auteur a également prouvé que la perturbation dans l'accumulation de l'amidon est causée déjà par l'intervention dans la synthèse des sucres, surtout de la saccharose. Exner (1958) a constaté que la Simazine a un effet inhibitif sur la réaction d'après Hill.

L'effet de la Simazine n'a pas encore été étudié dans la relation avec les viroses des plantes. Le but de notre travail était de vérifier expérimentalement les observations faites en juillet à Erfurt, dans la République démocratique allemande sur un champ sous des pommes de terre, sur lequel a été apportée par la pluie la Simazine appliquée en arrosage sur le champ voisin de maïs. Les plantes de la pomme de terre atteintes de viroses avaient péri, ou bien elles étaient gravement endommagées; mais les plantes sans symptômes de viroses n'ont point souffert de dommages.

Partie expérimentale

Nous avons fait nombre d'expériences en serre, tant avec les plantes du tabac (*Nicotiana tabacum* Samsun) saines et infectées par la souche Al du VTM (Blattný 1955), qu'avec les plantes de l'alsine *(Stellaria media)* saines et atteintes de la jaunisse, proche de l'aster yellows. Les plantes du tabac choisies pour les expériences avaient de quatre à cinq feuilles. Ces plantes étaient cultivées en pots mis dans les boîtes de Pétri; on arrosait la terre. On a expérimenté avec plusieurs concentrations de Simazine; en tant qu'optimale s'est avérée la concentration de 10 *μg*/ml et 6,6 *μg*/ml (dilution de 1 : 100 000 et de 1 : 150 000). Chaque plante a été arrosée avec 50 ml de la solution respective, les témoins en même nombre que les plantes expérimentales étant arrosées avec le volume d'eau correspondant. Au cours de l'évolution ultérieure de l'expérience, les plantes ne furent plus arrosées qu'avec de l'eau. Les expériences furent entreprises en automne 1959, au printemps et en été 1960. La durée de chaque expérience était de cinq à huit semaines.

Résultats et discussion

Les résultats des expériences individuelles sont groupés dans le tableau ci-dessous.

De ce tableau il s'ensuit que l'arrosage avec de la Simazine en concentrations citées de la solution d'arrosage, cause un dépérissement des plantes malades. Les premiers symptômes du dépérissement, soit la dessiccation des

Tableau 1. L'effet de la Simazine sur les plantes saines et sur celles atteintes de maladies à virus

Jour de l'arrosage	Plante*)	Variation expérimentale	Dose de Simazine pour une plante	Nombre de plantes mortes	= p . 100
12. 8. 1959	*Nicotiana tabacum* Samsun	saines malades	0·5 mg	0 10	0 100
19. 8. 1959	*Nicotiana tabacum* Samsun	saines malades	0·33 mg	0 10	0 100
31. 3. 1960	*Stellaria media*	saines malades	0·5 mg	6 30	20 100
31. 3. 1960	*Stellaria media*	saines malades	0·33 mg.	0 24	0 80
16. 5. 1960	*Nicotiana tabacum* Samsun	saines malades	0·33 mg.	0 12	0 60

*) Les témoins arrosés d'eau croissent normalement et l'air des plantes saines était le même que celui des plantes saines arrosées avec de la Simazine.

feuilles dès la base, commencent à se manifester environ le dixième jour après l'arrosage. Le cliché N° 1 montre les plantes saines et les plantes malades, arrosées avec de la Simazine, au moment de la fin des expériences. Les plantes saines manifestent après l'arrosage des symptômes d'une faible chlorose. Ces symptômes ne se manifestent qu'en automne ou tôt au printemps, tandis qu'ils n'étaient pas constatés au cours des expériences faites pendant la période de la prolongation de la journée (fin de mai et début de juin). Comme on le voit sur le tableau, l'effet de la Simazine sur les plantes atteintes de viroses dépend probablement de la durée de la photopériode. Ces phénomènes peuvent être en accord avec les connaissances actuelles sur le mécanisme de l'action de la Simazine. Si la Simazine intervient destructivement dans la biosynthèse des glycides, l'influence de la durée et de la qualité de la lumière, nécessairement doit s'y faire valoir.

Pourquois seules les plantes atteintes de viroses dépérissent-elles après l'arrosage avec de la Simazine c'est une question qui reste ouverte. On ne sait pas encore, de quelle manière générale le virus influence la plante-hôte, et nos observations sur la pomme de terre, sur le tabac et sur l'alsine, de même que les observations préliminaires du houblon faites par POZDĚNA, indiquent qu'il faudrait chercher un facteur commun peut-être pour toutes les plantes atteintes de viroses. Nous sommes de l'opinion qu'une étude approfondie de cette synergie dans l'effet de la Simazine, éventuellement dans d'autres herbicides, et dans l'effet des virus attaquant les plantes, pourrait contribuer non seulement à mieux connaître le mécanisme de l'action de la Simazine, mais aussi a élucider les questions plus importantes que le sont les changements dans les processus métaboliques produits par les virus dans

Cliché No 1: *Stellaria media* après l'arrosage avec de la Simazine. A gauche, plante saine, à droite, plante malade. Arrosage effectué le 31. 3., prise du cliché: 4.5., au moment de la fin de l'expérience.

les plantes-hôtes. La connaissance de cette synergie donne également de nouvelles possibilités de travail pour la solution des questions sur l'état latent des viroses.

Remerciment: Nous remercions A. Petrovská et Z. Procházková de leur assistence technique.

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Синергическое действие Симазина и вирусного заболевания растений как метод выявления вирусного заболевания

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При изучении действия Симазина на растения зараженные вирусом было обнаружено, что полив раствором Симазина в концентрации не оказывающей никакого вредного действия на здоровые растения (0,33 мг или 0,50 мг на 1 растение), влечет за собой гибель растений зараженных вирусом. Такой синергизм действия Симазина и вирусного заболевания наблюдался у растений табака зараженных вирусом табачной мозаики, у звездчаток больных желтухой близкой к *aster yellows*, на растениях картофеля и предварительные данные имеются также и о больных растениях хмеля.

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Electron Microscopic Investigation of Cytoplasm of Scutellar Cells of a Young Wheat Embryo

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Elektronově mikroskopické studium cytoplasmy štítkových buněk mladého embrya pšenice

V cytoplasmě štítkových buněk mladého embrya pšenice byly nalezeny tyto útvary: Paladeho granula a endoplasmatické reticulum, Golgiho aparát, lištovité membrány, šedá tělíska, hustá tělíska, silnostěnná tělíska, jemně granulovaná hmota, kapslovitá tělíska, ostře ohraničené vakuoly a struktura typu žloutkového jádra. Mezi tzv. aleuronová zrnka můžeme zařadit šedá tělíska. Byla diskutována tvorba, funkce a vzájemné vztahy těchto struktur.

Summary

The cytoplasm of scutellar cells of a young wheat embryo was found to contain the following structures: Palade granules, endoplasmic reticulum, Golgi apparatus, lath membranes, grey bodies, dense bodies, thick-walled bodies, fine granulated mass, capsulated bodies, sharply contoured vacuoles and structure of the "yolk nucleus" type. The formation, function and possible relationships between these structures are discussed.

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Introduction

The ultrastructure of scutellar cells of a young wheat embryo had not been studied so far. SETTERFIELD, STERN and JOHANSTON (1959) investigated the fine structure of cells of mature germinating wheat and pea embryos. Using an optical microscope, the scutellar cells of a young immature embryo of wheat were found to contain a number of inclusions which were very difficult to distinguish (Fig. 1). It was attempted therefore to differentiate between these inclusions by examining the ultrastructure with the aid of an electron microscope.

Material and Methods

Wheat embryos (*Triticum vulgare*, Stupická variety) were extirpated from young seeds during the time of milk ripeness, fixed in buffered OsO_4 (BUVAT 1958) and dehydrated with alcohols to be embedded in a mixture of methacrylates (93 parts of butyl and 7 parts of methyl methacrylate) according to SITTE (1958). Polymerization was carried out by adding 1% benzoyl peroxide at 60° and leaving overnight. Ultra-thin sections were cut on a Niklowitz ultramicrotome. Grey and silver sections were picked up by the usual technique and examined and photographed on a new type of Tesla electron microscope (Institute for Apparatus Technology, Czechoslovak Academy of Sciences, Brno) and on a RFT microscope (Berlin Oberschöneweide). The thicker control sections were stained with haematoxylin and examined under an optical microscope (Fig. 1).

Results

Ergastoplasm

Parallelly organized membranes (endoplasmic reticulum — ER) occur rarely (Fig. 7c). Palade granules are either freely scattered in the cytoplasm or occur in clusters (Figs. 4, 6).

Elongate cavities may be observed among the Palade granules (Fig. 4). In addition to these structures a special type of membranes can be found which will be described later (see the section of thick-walled bodies). Invaginations of the ectoplasmic pellicle (I) described by BUVAT 1958) occur at the periphery of cells (Fig. 2).

Structure of the "yolk-nucleus" type

A structure resembling some types of "yolk nucleus" of animal cells (YN) was found in the ergastoplasm among other structures described here (Fig. 5a,b). It is a circular area composed of double membranes of spindle arrangement, running parallelly through the entire structure. The outer membranes enclose the inner parts which contain layered clusters and cisternae with a vesicular termination. Structures of a similar type have been described from oocytes of some animal objects (REBHUN 1956) as yolk nuclei. The yolk nuclei form a part of basophilic structures of oocyte cytoplasm.

Golgi Apparatus

The Golgi apparatus (GA) was found in the form of so-called dictyosomes known from plant cells (Fig. 3, 5a, b).

Mitochondria

Some mitochondria (M) differ in their form from mitochondria found in plant material. SITTE (1958) calls attention to the fact that plant mitochondria do not possess the inner structure of the type usual with higher and lower animals (cristae mitochondriales, tubuli mitochondriales). He found vesicular structures which represent a transition between the cristae mitochondriales and the tubuli mitochondriales. SITTE calls them sacculi mitochondriales. In the object described here the shape of some of the inner mitochondrial structures is ring-like (Fig. 6) rather than vesicular (ring-shaped cristae).

Reserve substances

Among the structures forming the reserve substances of scutellar cells of wheat embryo the following three main types have been established:

Grey bodies (GB) and their derivatives

The grey bodies are weakly osmiophil structures, probably of protein character (Fig. 7a, 8—12). They resemble morphologically the protein products found in animal cytoplasm (yolk granules, zymogen granules etc.). In addition to spherical structures some structures of a similar character but irregular (Irregular shaped mass — IM), as were described by SETTERFIELD, STERN and JOHNSTON (1959) and others (Fig. 2,6). The spherical shape of the grey bodies occurs in cells in which the cytoplasm is shrunk, apparently due to the effect of dehydration accompanying the maturation of embryos. STRUGGER (1957) described a similar shrunk structure in the root meristem of *Allium cepa* during the dormancy (dry seeds). The possibility of shrinkage of cytoplasm due to unsatisfactory fixation is not considered here. Occasionally the irregular mass (IM) can be observed in connection with accumulated Palade granules. Sometimes the irregular mass contains very fine osmiophil granules (FM), sometimes arranged in regular rows (Figs. 2, 6, 7b) The irregular mass may also contain small vacuoles.

Dense bodies (DB) and their derivatives

Osmiohil bodies of apparently lipid character (Fig. 3—5) are mostly of irregular shape and frequently in a close connection with the protein irregular mass (IM). Sometimes it is difficult to distinguish between them (Fig. 5) and it is not sure whether we are always dealing here with different structures (the effect of section thickness and irregularity of staining with OsO_4)

Thick-walled bodies (TB) and their derivatives

In some cells the ergastoplasm is observed to contain membranes running in different directions surrouding the grey bodies and special vacuolar

Symbols used in Figs. 1—12.

IM — irregular mass, MM — membranous mass, FM — fine granulated mass, PL — plasmodesmas, I — invaginations of ectoplasmic pellicle, CHR — chromosomes, M — mitochondria, GA — Golgi apparatus, V — vacuoles, DB — dense bodies, CM — cell membrane, YN — structure of the "yolk nucleus" type, GB — grey bodies, LM — lath membrane, SV — sharply contoured vacuole, ER — endoplasmic reticulum, TB — thick-walled bodies and their precursors, C — capsu'e of the capsulated body, CI — central inclusion, LI — layered central inclusion, CB — capsulated bodies, NL — nucleolus, N — nucleus, VI — different types of inclusions and vacuoles.

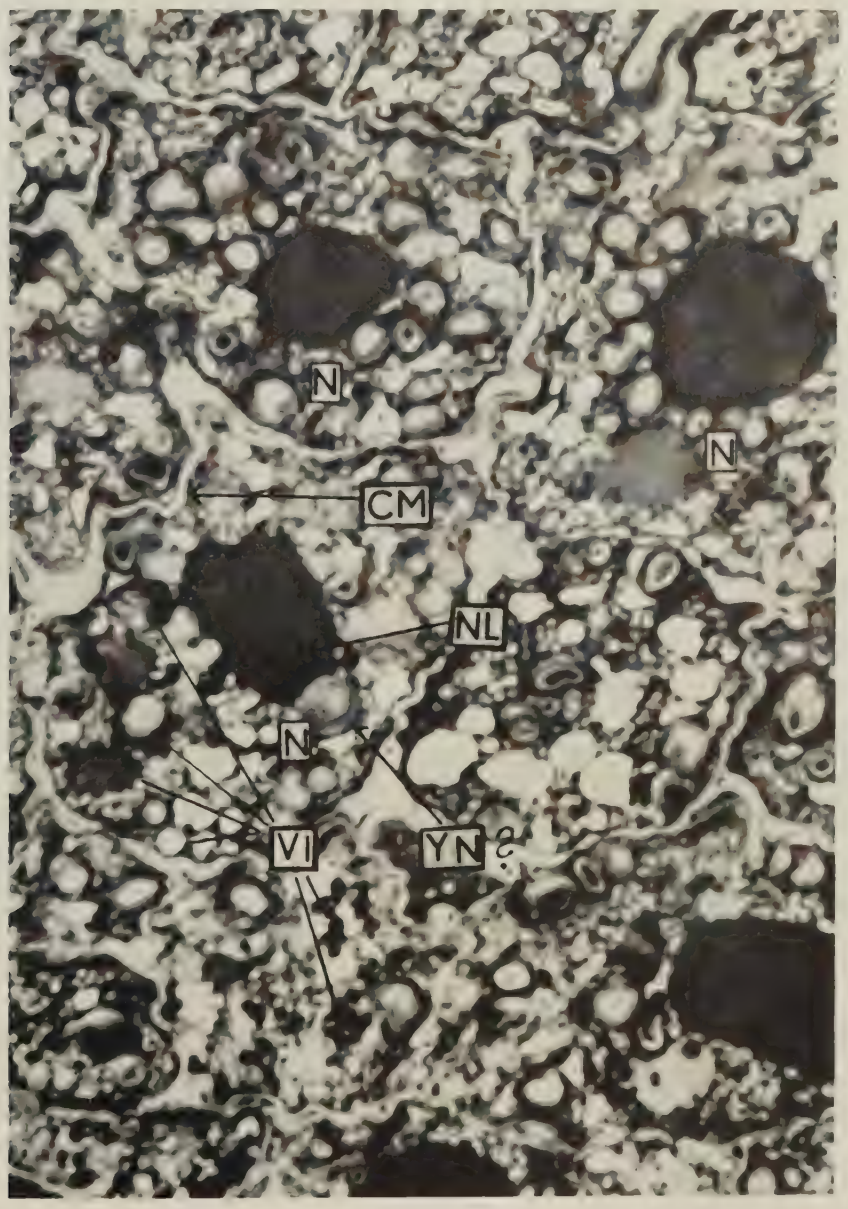

Fig. 1. Scutellar cells of a young wheat embryo. Fixed in buffered OsO_4 according to Buvat, methacrylate-embedding. Stained with Regaud's haematoxylin. Optical microscope, direct magnification 900×, total 2000×.

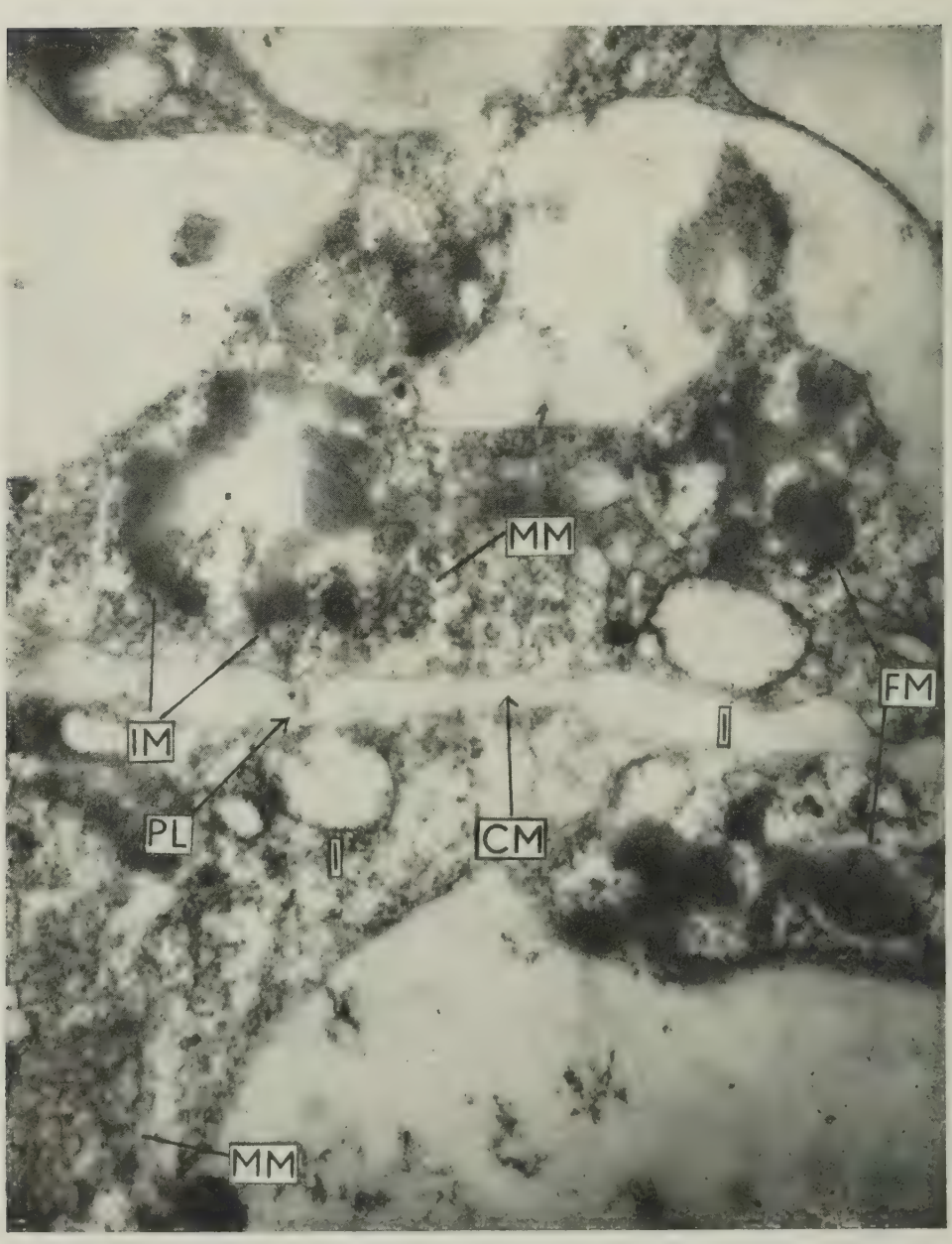

Fig. 2. Cytoplasm of a scutellar cell of an immature wheat embryo. Irregular mass, fine granulated mass, invaginations of ectoplasmic pellicle and membraneous mass. Direct magn. 15 800×, total 31 600×.

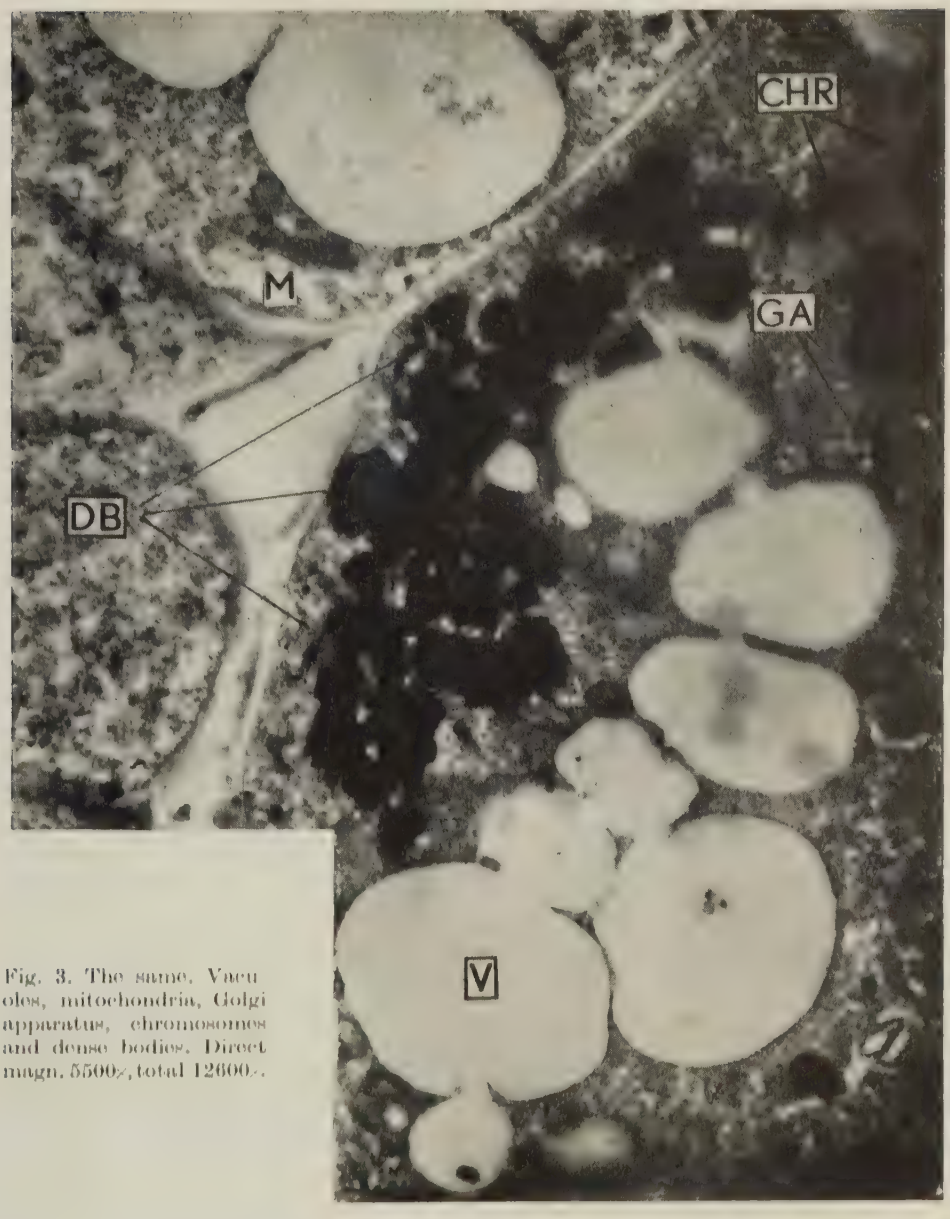

Fig. 3. The same. Vacuoles, mitochondria, Golgi apparatus, chromosomes and dense bodies. Direct magn. 5500×, total 12600×.

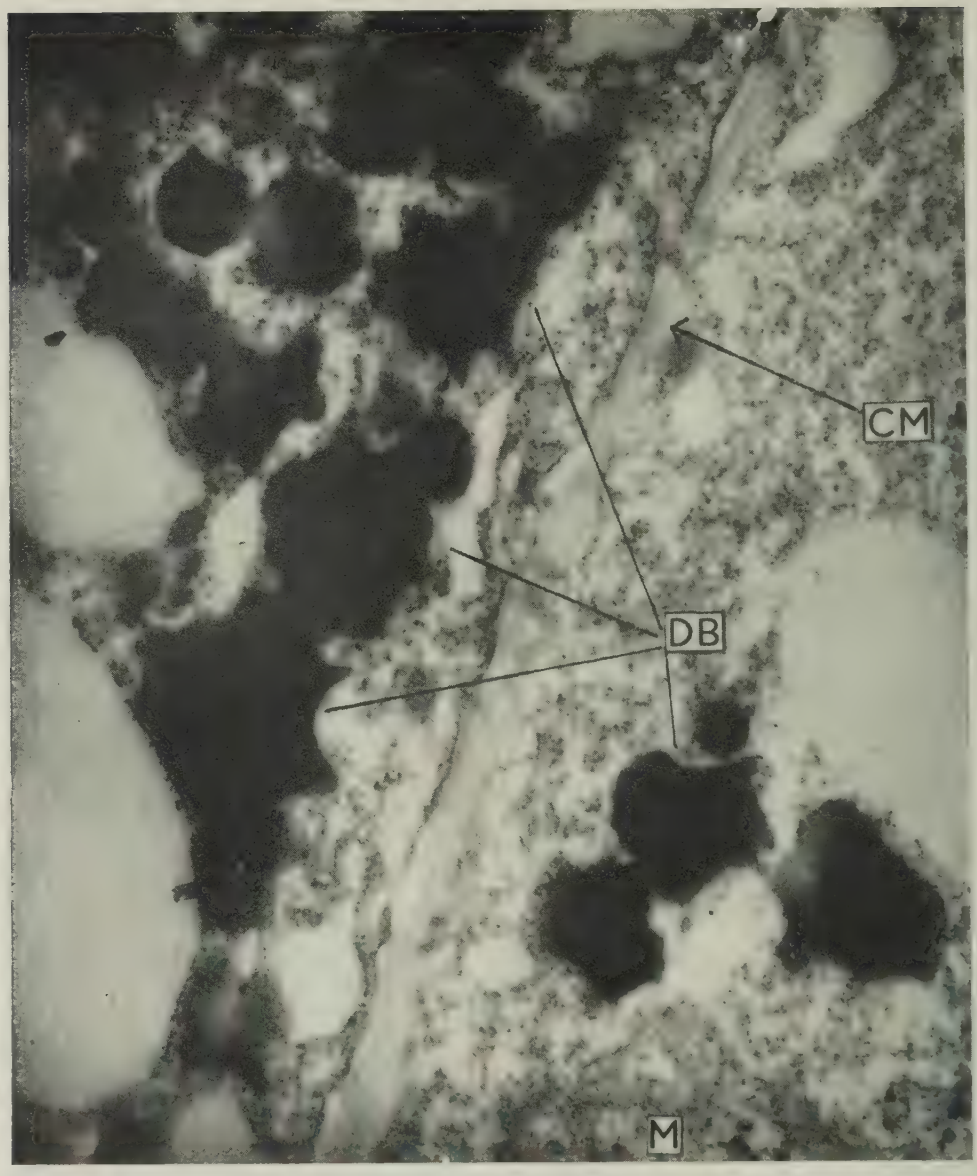

Fig. 4. The same. Dense bodies, mitochondria, cell membrane. Direct magn. 11 300 ×, total 27 000 ×.

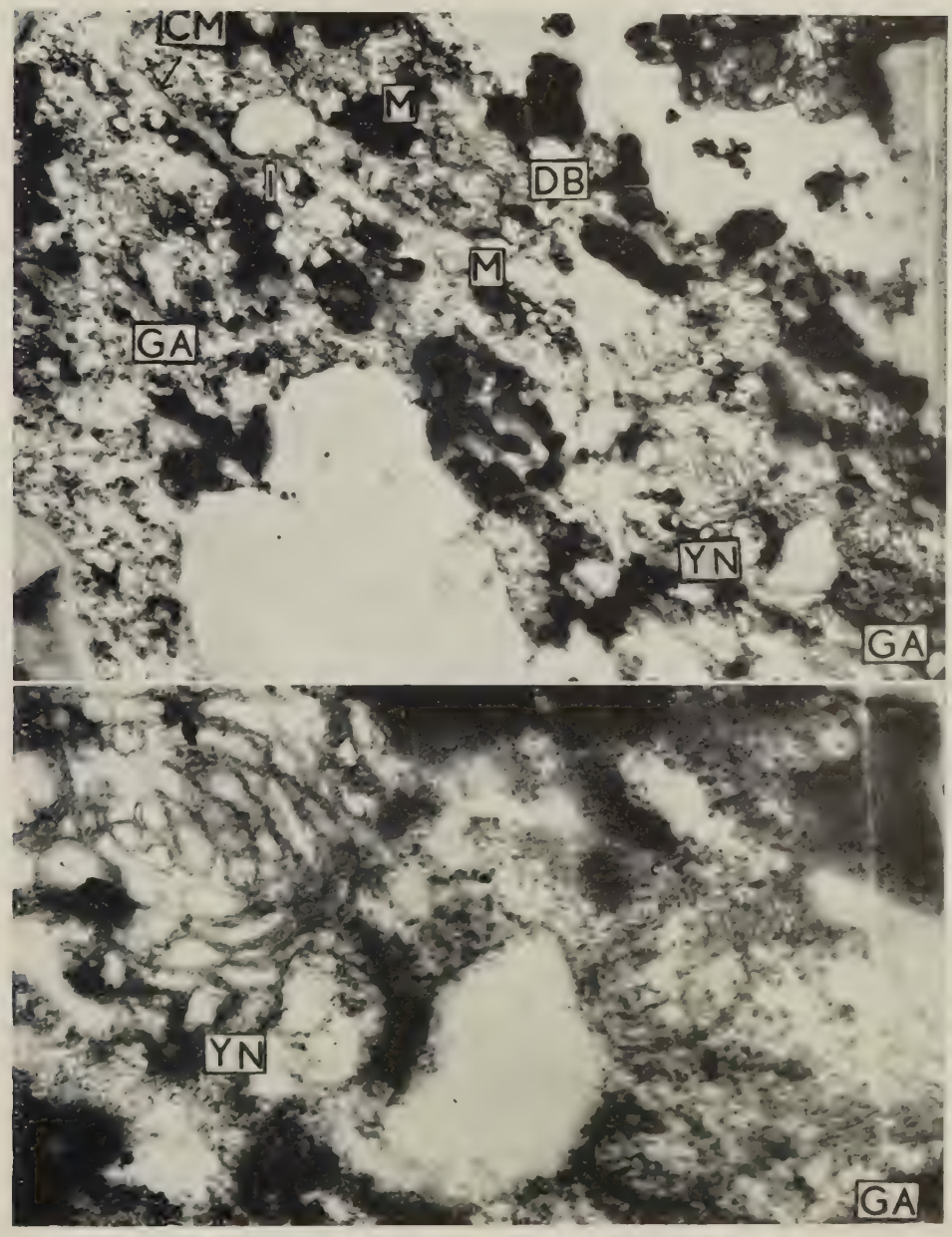

Fig. 5a. Cytoplasm with the structure of the "yolk nucleus" type, Golgi apparatus, irregular mass and mitochondria. Direct magnification 5500×, total 11 000×.

Fig. 5b. The same at a greater magnification. Direct magn. 5500×, total 27 500×.

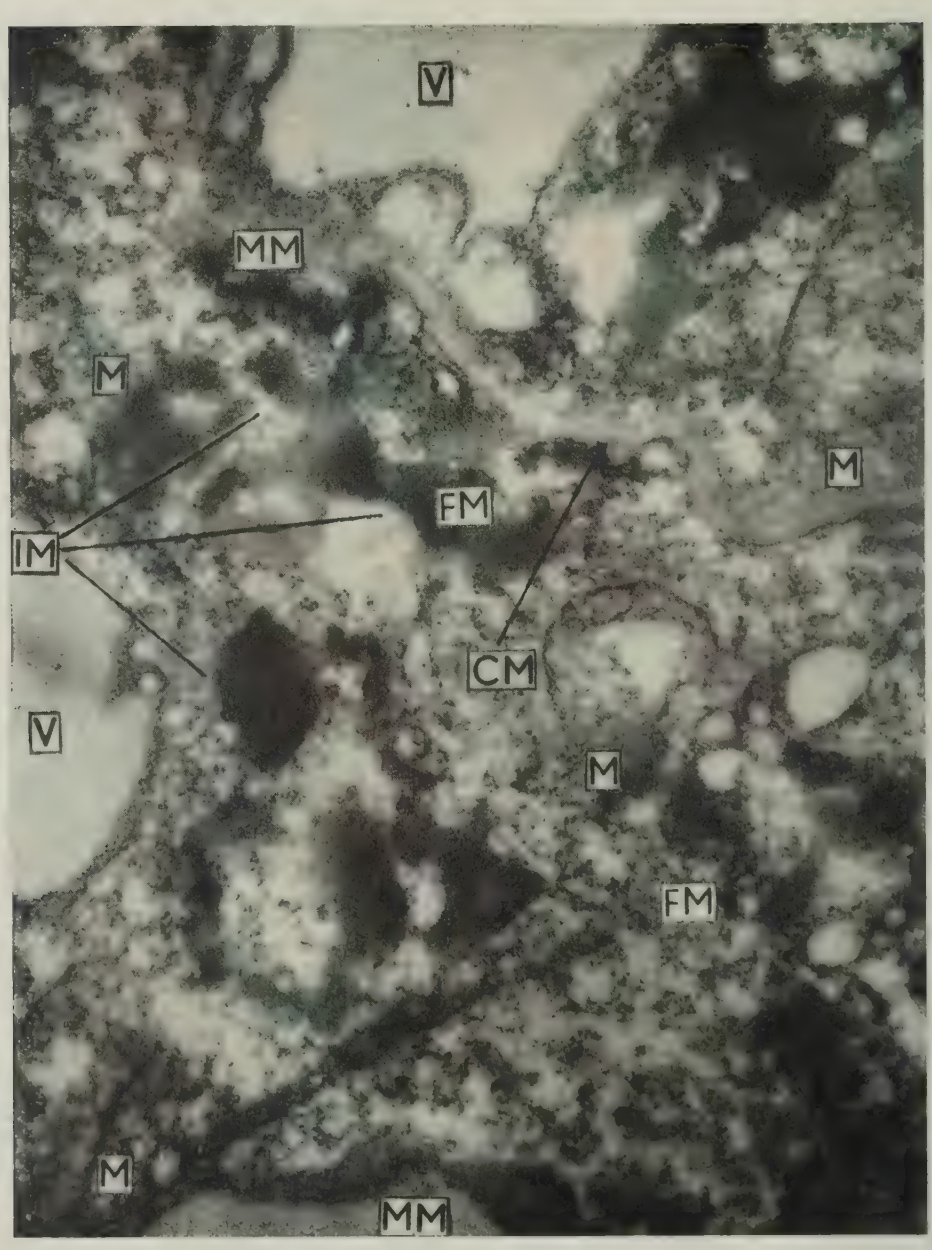

Fig. 6. Cytoplasm with mitochondria, irregular mass and fine granulated mass. Direct magnification 15 800×, total 35 500×.

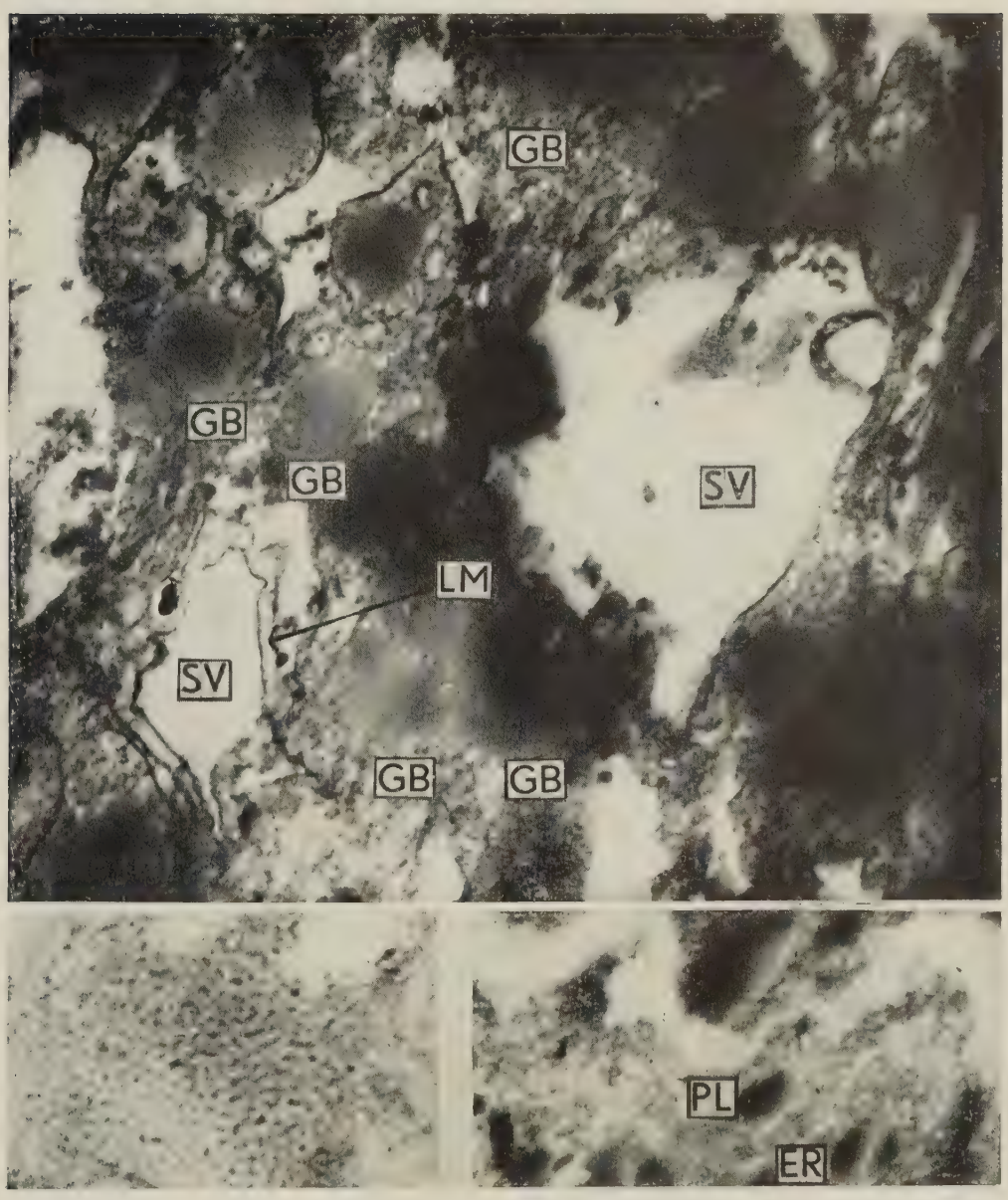

Fig. 7a. Cytoplasm with grey bodies, membranes and sharply contoured vacuoles. Shrinkage of cytoplasm. Direct magn. 22 500×, total 78 500×.

Fig. 7b. Fine granulated mass at high magnification. Direct magn. 15 800×, total 142 000×.

Fig. 7c. Endoplasmic reticulum with parallelly arranged membranes. Plasmodesmas. Direct magn. 6000×, total 19 800×.

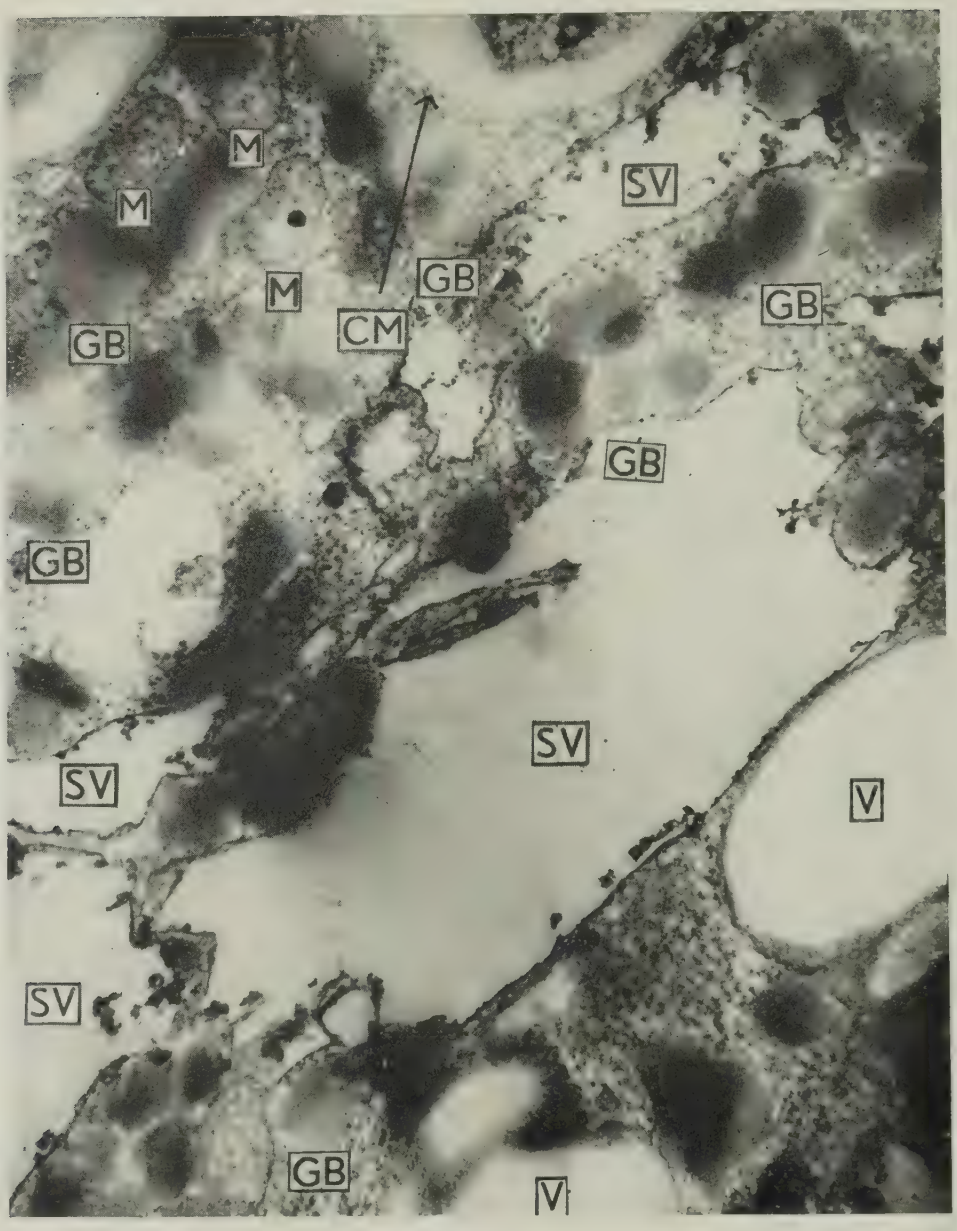

Fig. 8. Grey bodies, sharply contoured vacuoles, normal vacuoles and mitochondria. Direct magn. 11 300×, total 28 800×.

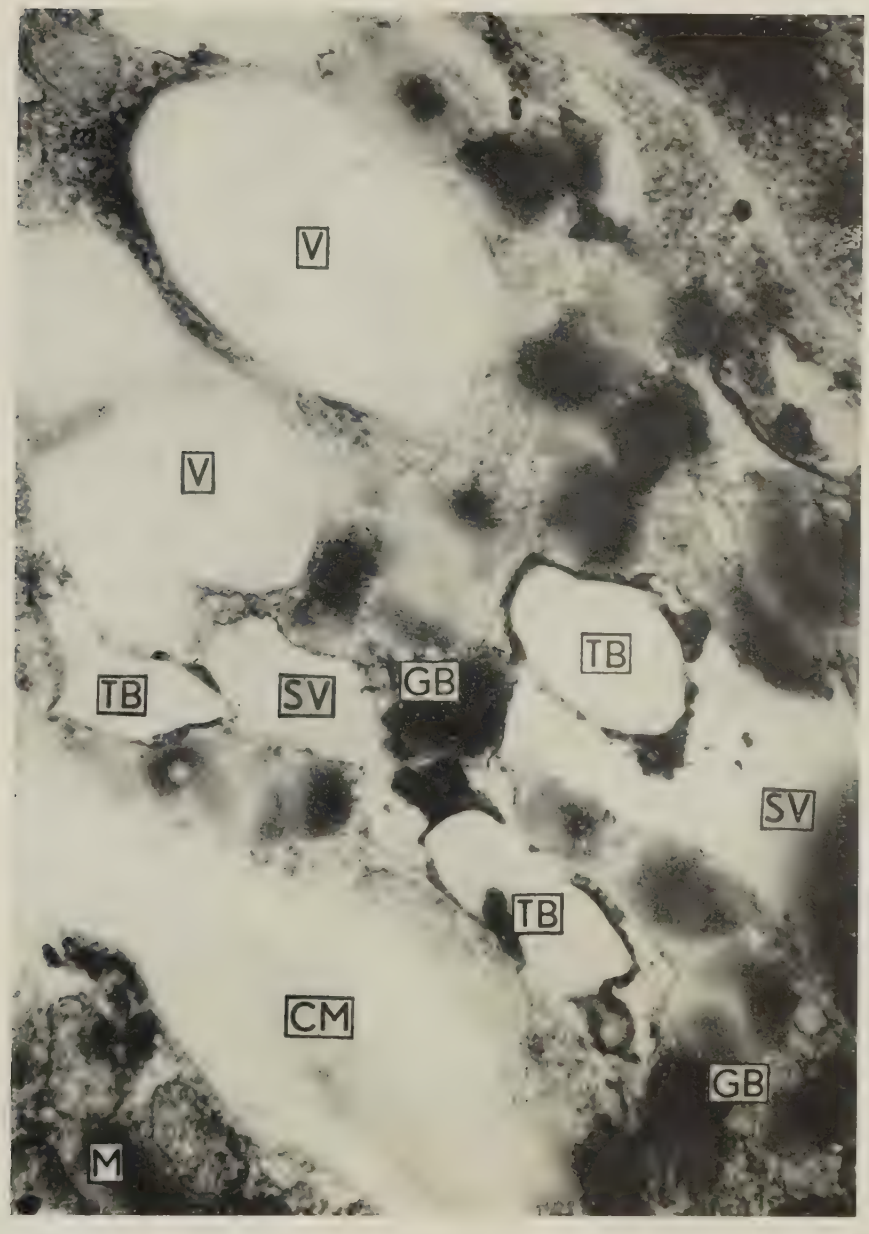

Fig. 9. Grey bodies, shraply contoured vacuoles, normal vacuoles and mitochondria. Direct magn. 11 300×, total 27 000×.

Fig. 10a. A group of capsulated bodies in a sharply contoured vacuole. Direct magn. 27 000 × total 33 700 ×.

Fig. 10b. Membrane of a sharply contoured vacuole. Direct magn. 22 500 × total of 28 ×.

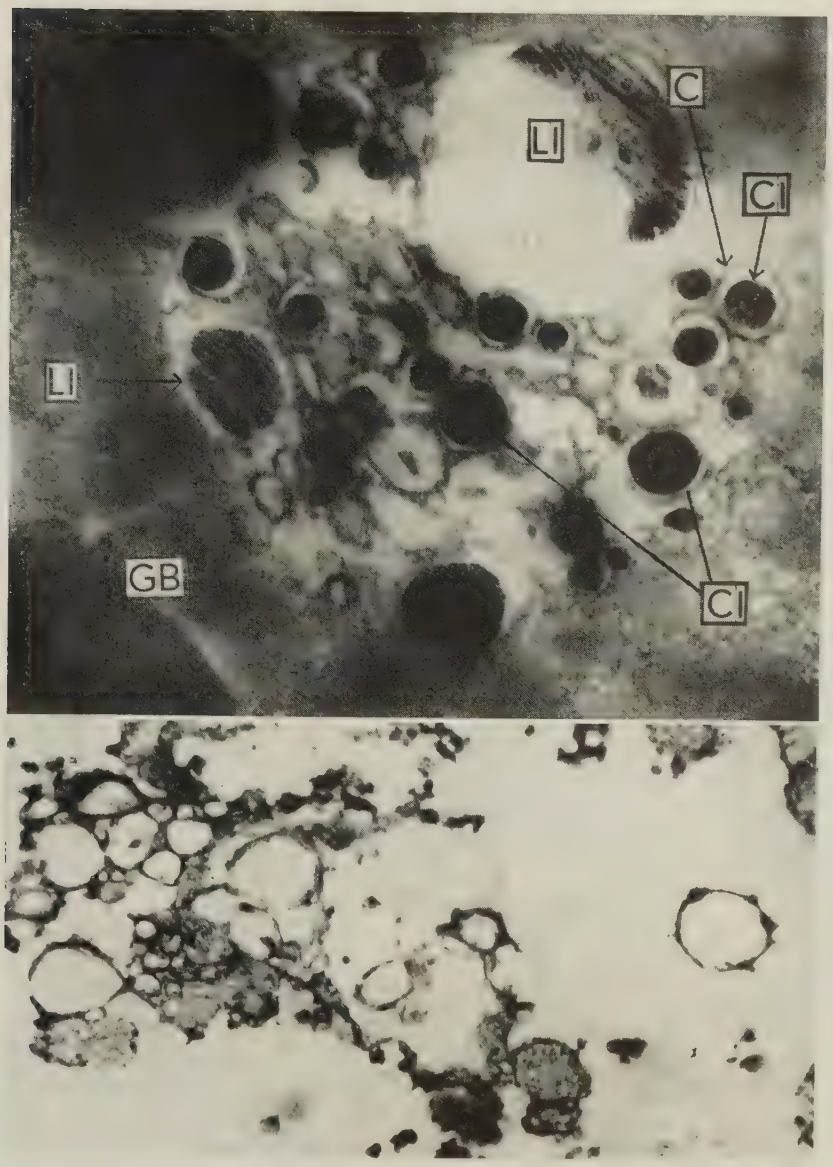

Fig. 11a. A group of capsulated bodies. Some of them possess rough layers of the central inclusion or minute vacuoles. Direct magn. 22 500×, total 34 800×.

Fig. 11b. Groups of sphaerical bodies bounded by membranes. Direct magn. 22 500×, total 45 000×.

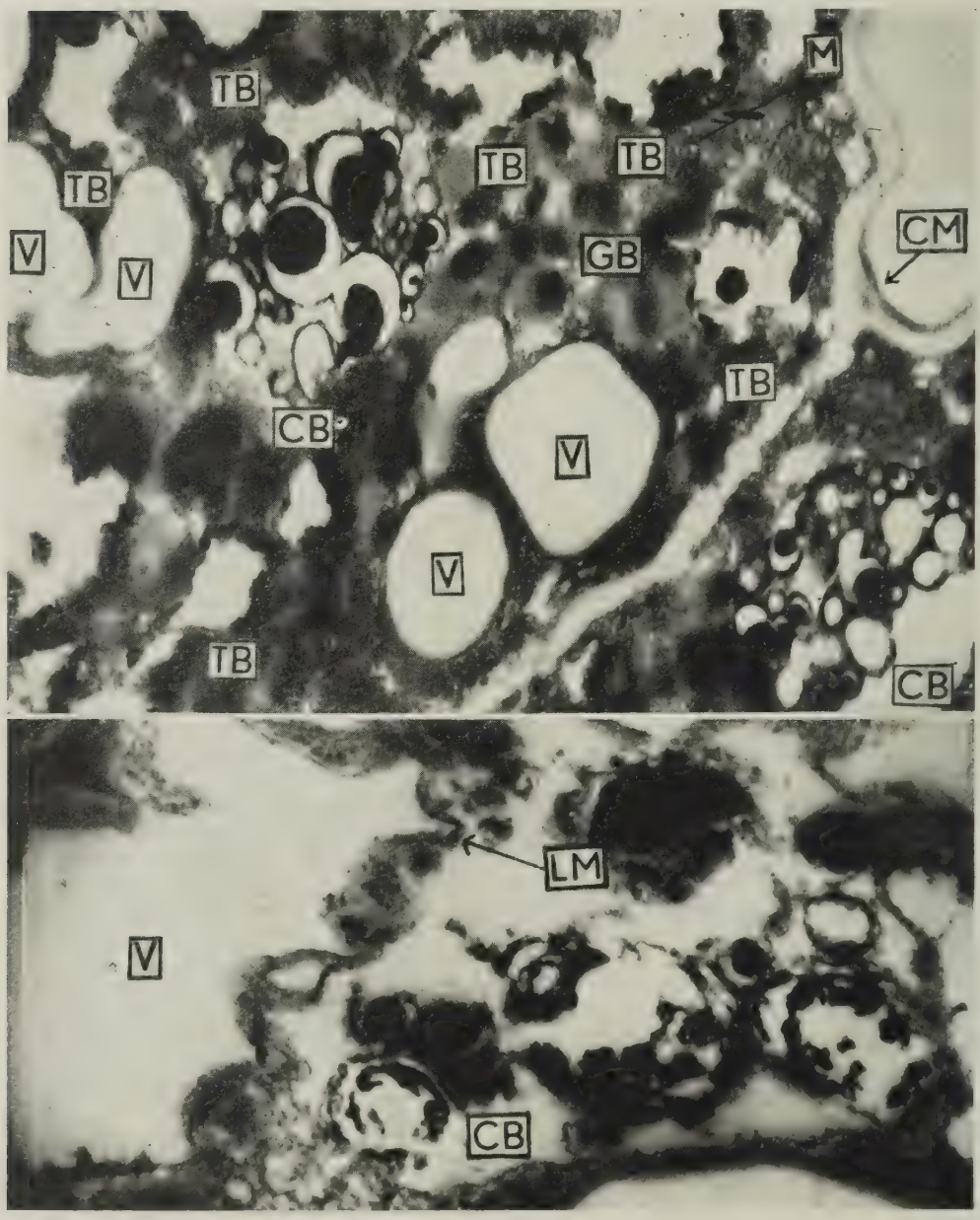

Fig. 12a. Part of cytoplasm with groups of capsulated bodies, thick-walled bodies, vacuoles and grey bodies. Direct magn. 6000×, total 18 000×.

Fig. 12b. Capsulated bodies with disintegration of the central inclusion. Direct magn. 22 500×, total 38 000×.

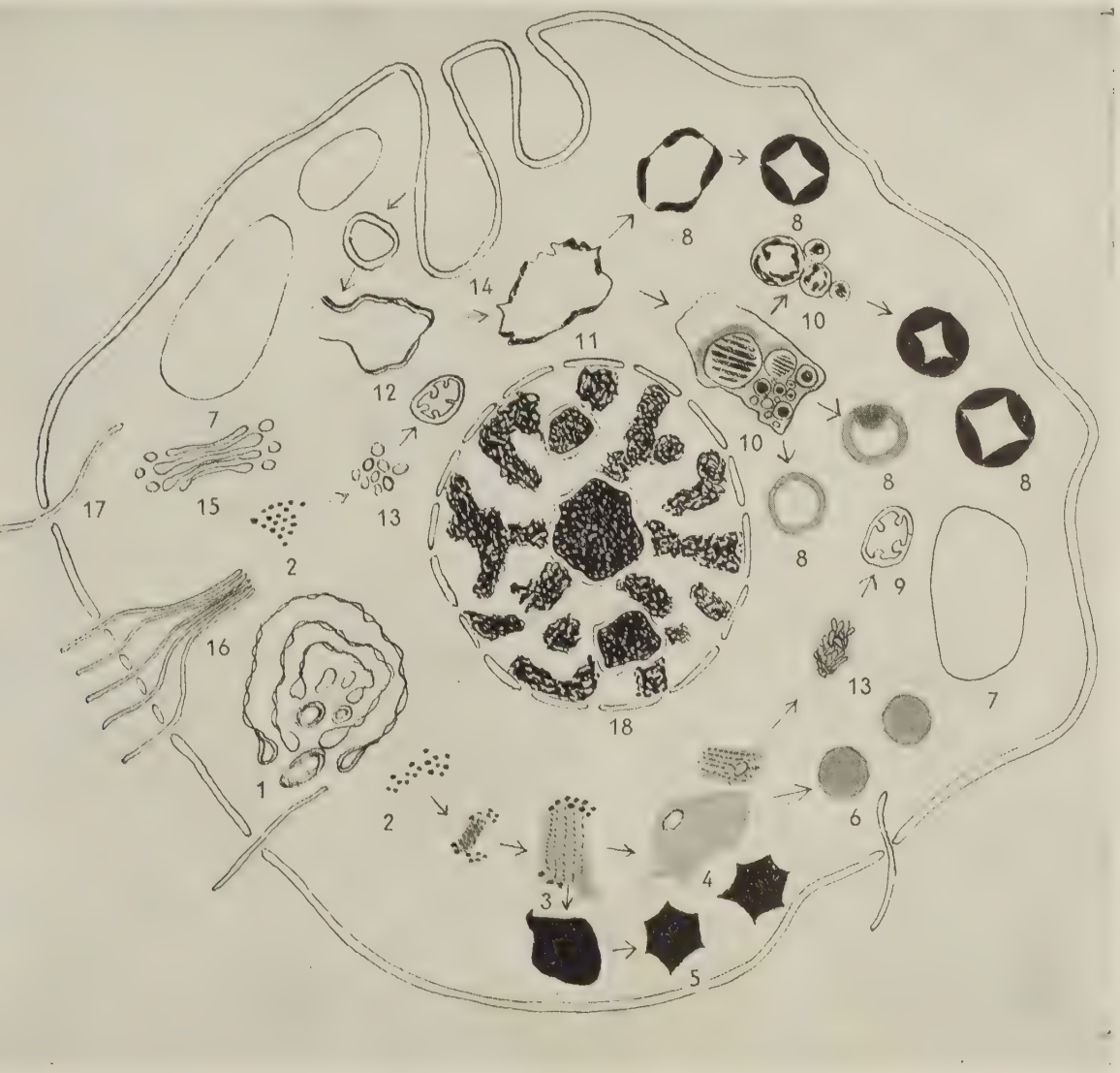

Fig. 13. Reconstruction of a scutellar cell of wheat with possible stages of development of the described structures.

Legend to Fig. 13

1. Structure of the "yolk nucleus" type (YN)
2. Palade granules
3. Fine granulated irregular mass (FM)
4. Irregular mass (IM)
5. Dense bodies (DB)
6. Grey bodies (GB)
7. Vacuole (V)
8. Different types of thick-walled bodies (TB)
9. Mitochondria (M)
10. Capsulated bodies (CB)
11. Sharply contoured vacuole (SV)
12. Lath membrane (LM)
13. Membranous mass (MM)
14. Invaginations of ectoplasmic-pellicle (I)
15. Golgi apparatus (GA)
16. Endoplasmic reticulum (ER)
17. Plasmodesmas (PL)
18. Nucleus (N)

structures (Fig. 7a — LM). It appears that these membranes differ from the normal double membranes of the endoplasmic reticulum (Fig. 10a, b). Both lines forming the membrane overlap at some places and one of them may be more osmiophil than the other (lath membranes). The membranes limit sharply some vacuolar structures (sharply contoured vacuoles — SV) and form numerous infoldings and invaginations around them (Figs. 7a, 8, 10). The sharply contoured vacuoles have an irregular shape and differ from the spherical or ovoid ones of the normal common type (Fig. 3, 8, 9 — V). The sharply contoured vacuoles contain irregular particles of strongly osmiophil material and probably phenolic nature (Fig. 8) and occasionally also structures or indications of structures described in the mature embryo as thick-walled bodies (Fig. 9, 12 — TB) by SETTERFIELD, STERN and JOHNSTON (1959). In the sharply contoured vacuoles occasionally larger or smaller groups of capsulated bodies (CB) may be found which are surrounded by a weakly osmiophil matrix (Fig. 10, 11a, 12a, b). The capsulated bodies consist of a weakly osmiophil capsule (C) which sometimes fades into the surrounding matrix and encloses an osmiophil inclusion (CI). The osmiophil inclusion sometimes contains small vacuoles. In the larger capsulated bodies parallel layers of the osmiophil mass occur (Fig. 10, 11a — LI) or else the osmiophil inclusions are disrupted to irregular clusters (Fig. 12b). At some places groups of spherical bodies of different size surrounded by a membrane of the same type as that surrounding the sharply contoured vacuoles may be observed. The inside of the bodies is empty (Fig. 11b). All these structures appear to belong to structures containing probably phenolic substances (the osmiophil substances in vacuolar spaces). Their formation, however, should be considered in connection with beginning dehydration accompanying the maturation of the embryo which is reflected in a shrinkage of other structures (Figs. 7a, 8—12).

Irregular membraneous mass

Among the cytoplasmic structures investigated formations may also be found which contain membraneous elements resembling inner mitochondrial structure (Fig. 2 — MM). These structures are also sometimes at the edges of vacuoles (Fig. 6).

Discussion

The present paper represents an attempt at distinguishing with the aid of an electron microscope, different types of nutritional substances occurring in the cytoplasm of a young wheat embryo. Some common structures have been found, such as e.g. ergastoplasm, mitochondria, Golgi apparatus and the normal type of vacuoles. The dense bodies, thick-walled bodies and irregular mass have also been described by SETTERFIELD, STERN and JOHNSTON (1959), the structure similar to the irregular membraneous mass by BUVAT (1959). Some structures have been found here, however, which had not been described from plants so far. For the sake of comparison a table

is presented containing the structures found by SETTERFIELD, STERN and JOHNSTON (1959) in a mature embryo and in this laboratory in an immature embryo:

Table 1.

Structures	Young immature wheat embryo	Mature germinating wheat embryo (SETTERFIELD et al.)
Palade granules	+	+
Endoplasmic reticulum	+	+
Lath-membranes	+	
Golgi apparatus	+	+
Mitochondria	+	+
Proplastids	—	+
Amyloplasts	—	+
Grey bodies	+	—
Irregular mass	+	+
Thick-walled bodies	+	+
Capsulated bodies	+	+
Sharply contoured vacuoles	+	+
Dense bodies	+	+
Fine granulated irregular mass	+	+
Membrane mass	+	
Structure of the yolk nucleus type	+	
Vacuoles	+	

The "yolk-nucleus" type structure (YN) indicates the presence of basophilic material resembling that of animal cells. It can represent, in common with animal cells, the site of formation of protein substances. The accumulation of Palade granules in some places near the irregular mass could also have connection with the activity of RNA-containing particles during protein synthesis of nutritive substances. The protein nutritive substances in cells of seeds and embryos were previouslly investigated in an optical microscope and described as the aleurons or aleuron grains. An electron-microscopic investigation reveals that their morphology is more complex than had been assumed hitherto. GUILLIERMOND (1941) and most plant cytologists thought on the basis of optical microscopy that aleuron grains originate from vacuoles and are stainable with neutral red during the first developmental stages. Electron microscopy points to the existence of several different types of structures. Perhaps only some of them, i.e. true vacuoles and thick-walled bodies can correspond to the inclusions which are stainable with neutral red. On the other hand, protein substances (grey bodies, irregular grey mass) are most likely formed directly in the ergastoplasm without any apparent relationship to any vacuoles.

The nutrient substances can be divided into three groups:

1. Weakly osmiophil granules (irregular mass, grey bodies, fine granulated mass) — IM, GB, FM.
2. Osmiophil granules (dense bodies) — DB.
3. Vacuolar inclusions (sharply contoured vacuoles, capsulated bodies, thick-walled bodies) — SV, CB, TB.

It is probable that the sharply contoured vacuoles (SV) and capsulated bodies (CB) are precursors of thick-walled bodies (TB) and may contain tannin-like and other phenolic substances.

Their origin can be related to extensive invaginations (I) of the ectoplasmic pellicle (Fig. 2) or of endoplasmic reticulum which apparently accompanies the shrinking of cytoplasm during maturation of the embryo (Fig. 7a, 8—12). The sharply contoured vacuoles (SV) and their derivatives differ morphologically from normal vacuoles (V) which occur together with the former (Figs. 8, 9).

The fine granulated irregular mass (FM) can represent a developmental form of protein (IM, GB) or lipid (DB) substances.

The membraneous structures of a similar shape as membraneous irregular mass (MM) have been described by Buvat (1959) in the vicinity of a forming fragmoplast during cell division. Buvat is of the opinion that we may be dealing here with mitochondria in the process of formation.

Acknowledgment:

The authors are indebted to Mr. V. Drahoš of the Institute for Apparatus Technology in Brno for kind preparation of electron micrographs on the new type of electron microscope, and to Mr. Fiala por preparation of the optical micrograph.

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Электронно-микроскопическое изучение цитоплазмы клеток щитка молодого зародыша пшеницы

BIOLOGIA PLANTARUM (PRAHA) 3 (2) : 126—130, 1961

В цитоплазме клеток щитка молодого зародыша пшеницы найдены: гранулы Палладэ и эндоплазматический ретикулум, аппарат Гольджи, багетовидные мембраны, серые тельца, плотные тельца, тельца с сильными стенками, мелко гранулированное вещество, капсуловидные тельца, четко ограниченные вакуоли и структуры типа желточного ядра. К называемым алейроновым зернышкам можно отнести серые тельца. Обсуждается образование, функция и возможные взаимоотношения этих структур.

BIOLOGIA PLANTARUM (PRAHA)

3 (2) : 132—136, 1961

The Electron Microscopic Examination of Inclusions of Cucumber Virus 4

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Elektronomikroskopická struktura inklusí viru zelenoskvrnité mozaiky okurky

Na ultratenkých řezech listem okurky infikované virem zelenoskvrnité mozaiky okurky (cucumber virus 4, *Cucumis* virus 2A) byly zjištěny inkluse složené z jedné vrstvy rovnoběžně uložených virových částic uspořádaných kolmo k dlouhé ose inkluse. Inkluse byly slabě barvitelné OsO_4 bez kontrastování. Délka částic odpovídala délce částic uvolněných homogenisací listu (2757 Å), avšak tloušťka částic pozorovaných v inklusi byla menší než na preparátech jednotlivých částic. Inkluse leží v cytoplasmě, nemá blánu, avšak je obklopena nepravidelnou, nebarvitelnou vrstvou. Po použití pufrovaného roztoku OsO_4 nebylo v okolí inkluse pozorováno porušení základní cytoplasmy, mitochondrií a lysosomům podobných útvarů. Inkluse se podle elektronogramů podobají inklusím viru mozaiky tabáku.

Summary

Ultrathin sections of cucumber leaves infected by cucumber virus 4 (*Cucumis* virus 2A) shoved inclusions composed of one layer of virus particles with parallel arrangement at right angles to the long axis of the inclusion. The inclusions were slightly stained by osmium tetraoxide without contrasting. The lenght of the particles corresponds to the lenght of individual particles obtained by homogenisation (2757 Å), but their thickness appears to be less than from preparations of individual particles. The inclusion lies in the cytoplasm, it has no membrane but is surrounded by an irregular non-stainable layer. After treatment with buffered O_8O_4 solution no disturbance of the basic cytoplasm, mitochondria, lysosome-like structures, and similar bodies was observed in the infected cells. According to electron microscopic observations inclusions of cucumber virus 4 bear a close resemblance to those of tobacco mosaic virus.

Introduction

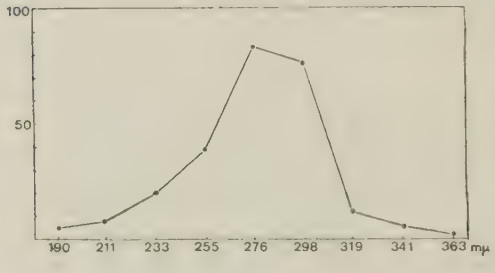
Particles of cucumber aucuba mosaic virus (cucumber virus 4, *Cucumis* virus 2A, *Marmor astrictum* var. *aucuba*) were first described by STANLEY and ANDERSON (1941). These authors established their similarity to tobacco mosaic virus. KNIGHT and STAŇLEY (1941a, b) measured the size of the particles of this virus (rod-like particles 275 × 15 mμ) and determined the biochemical differences as compared with tobacco mosaic virus. Some later reasearch (FULTON 1950, KNIGHT 1955) has confirmed that the virus is specifically distinct from the tobacco mosaic virus, although it has many properties similar to those of the latter. The first report on cytoplasmic inclusions of this virus in cucumber was published by VOVK (1948), later GOLDIN (1954) also mentioned them. Microscopic examination by the present authors showed a small number of crystalline inclusions in the hair cells of infected cucumbers (needle-shaped inclusions, 2—12 μ in length, usually 5 μ, and hexagonal crystals 4—22 μ in length, usually 11 μ, and 1—9 μ wide, usually 3 μ); Fig. 1. They were scarcely distinguishable from the crystalline inclusions in the cells of healthy cucumbers (Fig. 2); however, the recommended methods for the specific staining of virus inclusions did not produce results enabling a cytological identification of the virus to be made. So far only SMITH (1954) has studied this virus in ultrathin tissue sections and he observed only a haphazard distribution of the rods in the infected cells. The fact is that the method of dissolving the methacrylate after the sections were prepared and shading the preparation became popular. Although this method was also applied, for example, for the estimation of the number of virus particles of tobacco mosaic virus in a cell (NIXON 1956), and although significant cytopathological findings were obtained by its use with the tobacco mosaic virus (BRANDES 1956), the method was shown to cause artefacts. SMITH (1956) shows in the case of tomato bushy stunt virus that after removal the methacrylate and shading only haphazard spherical particles are visible in the sections, while in sections from which the methacrylate was not removed and which were not shaded a regular crystalline arrangement of the particles appears. Artificial distribution of tobacco mosaic virus rods in ultrathin tissue sections was also observed by GOLDIN and FEDOTINA (1956). For this reason SHALLA (1959a) abandoned the original method and achieved better results by staining the tissue with phosphotungstic acid. With this method, however, damage is caused to the surrounding structure, apparently due to the low pH of phosphotungstic acid (WILLIAMS 1957).

These problems of methods were important in the present work, in which a comparison is made of examination using ultrathin tissue sections and preparations made from individual virus particles. The aim was to determine the localisation of virus particles in the infected cells of cucumber leaves and their identification by comparison with preparations of individual particles. It was also intended to provide proof of the existence of virus inclusions in the cells of infected plants while preserving the cytoplasmic structure.

Material and Methods

The virus was obtained by the kind help of Dr. Uschdraweit from Berlin (it was a yellow strain — Fig. 3). Preparations of individual particles were made by grinding 0·5 cm² of leaf edge (from cucumbers infected in the glasshouse) in 10 ml. of redistilled water and the suspension was spred on grids covered with collodion membrane. Shading was carried out with Au + Pd and carbon. The preparations were photographed with a Tesla table electron microscope simultaneously with latex balls of diameter 1880 A. Measurement of the lengths of the virus rods was made after drawing their outlines and those of latex balls using a projection apparatus (total magnification of 100 000 times). In all 253 particles were measured. The length of the particles was determined according to the average value of the simultaneously measured latex.

For preparation of the ultrathin tissue sections young leaves (2—3 cm. in length) were fixed with buffered OsO_4 solution according to Buvat (1958), dehydrated with alcohol and transferred to a mixture of 93 parts of butylmethacrylate and 7 parts of methylmethacrylate (Sitte 1958). Polymerisation was carried out after addition of 1% benzoylperoxide at 60 °C. Sections were made on a Niklowitz ultramicrotome; silver and grey sections were picked up on specimen grids covered with a formvar membrane. For the examination and photography a new Tesla electron microscope at the Institute of Instrumental Technique in Brno was used.



Graph: Variability of length of isolated cucumber virus 4 particles in millimicrones, *Y* number of particles.

Results and Discussion

Measurement of the length of virus rods from homogenates gave an average length of 2757 Å (Fig. 4), which corresponds to the first measurement ever made of this virus (Knight and Stanley 1941a) and is somewhat less than that given by Procenko and Nesterova (1955).

In spite of the known poor staining of plant viruses with OsO_4 (Williams 1957), it was possible in ultrathin tissue section to distinguish elongated, angular inclusions composed of rod-shaped virus particles aggregated at right angles to the longer axis of the inclusion so that the thickness of the inclusion corresponded to the length of an individual particle. In view of the unsatisfactory staining of the virus with osmium tetraoxide, however, the possible occurrence of particles in the cytoplasm in addition to the inclusions cannot be excluded. On the other hand, the above method enabled structural cell details to be preserved, while these are destroyed or disturbed by the methods mentioned in the introduction. Accompanying pathological changes of the chloroplasts will be described later by the second of the present authors. Inclusions (Figs. 5, 6, 7) lie in cytoplasm which contains Palade granules. They have no covering membranes, as was also found by Wehrmeyer (1957) in the case of tobacco mosaic virus, but longitudinally (at both ends of the grouped rods) there is an irregular osmiumphobic layer. Alongside the inclusion there are Palade granules in the basic cytoplasm, mitochondria, and lysosomelike bodies (dense bodies) (Genevés, Lance, Buvat 1958). Neither of these formations would appear to be damaged. The cytoplasm is bounded

by an ectoplasmic pellicle; in the central vacuole there are round formations evidently containing Palade granules and further vesicular and membranic elements.

In the parallel arrangement of its particles the cucumber virus 4 inclusion closely resembles the tobacco mosaic virus inclusions as seen in ultrathin tissue sections by Nixon and Sampson (1954), Wehrmeyer (1957) and others. In the present case, however, we are concerned only with a single layered band, similar to the inclusions discovered by Shalla (1959b) in embryonic tobacco leaves infected with tobacco mosaic virus. The similarity of these inclusions to inclusions of tobacco mosaic virus (older leaves) is also to be found in the fact that they lie near the cell periphery, close to the cell wall. In the present case, however, the formations are smaller and simpler, not clearly distinguishable with an optic microscope.

The length of particles in an inclusion corresponds to the length of particles freed by grinding of infected leaf tissue. However the thickness of the virus rods, in so far as this can be roughly judged in the tissue sections from the measurement of a number of closely packed particles, seems to be less than obtained by measuring roughly shaded individual rods.

Acknowledgment:

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Электронно-микроскопическая структура клеточных включений *Cucumis* virus 2A.

BIOLOGIA PLANTARUM (PRAHA) 3 (2) : 132—136, 1961

На сверхтонких срезах через лист огурца, инфицированного вирусом cucumber virus 4 (*Cucumis* virus 2 A), были обнаружены клеточные включения, состоящие из одного слоя параллельных вирусных частиц, расположенных перпендикулярно по отношению к длинной оси включения. Включения слабо окрашивались осмиевой кислотой без контрастирования. Длина частиц соответствует таковой у частиц, выделенных из тканей путем гомогенизации (2757 Å), но толщина кажется меньше, чем на препаратах отдельных частиц. Включения находятся в цитоплазме, не имеют оболочек, но окружены нерегулярным, неокрашивающимся слоем. После применения осмиевой кислоты с буфером около включения не наблюдалось нарушения основной цитоплазмы, митохондрий и образований, похожих на лизосомы. Включения cucumber virus 4 по электронограммам очень похожи на включения табачной мозаики.

Fig. 1. Crystalline inclusions in hair cells of cucumber infected by cucumber virus 4.

Fig. 2. Crystalline inclusions in hair cells of healthy cucumber.

Fig. 3. Leaves of cucumber var. Delikates showing symptoms of systemic infection by cucumber virus 4.

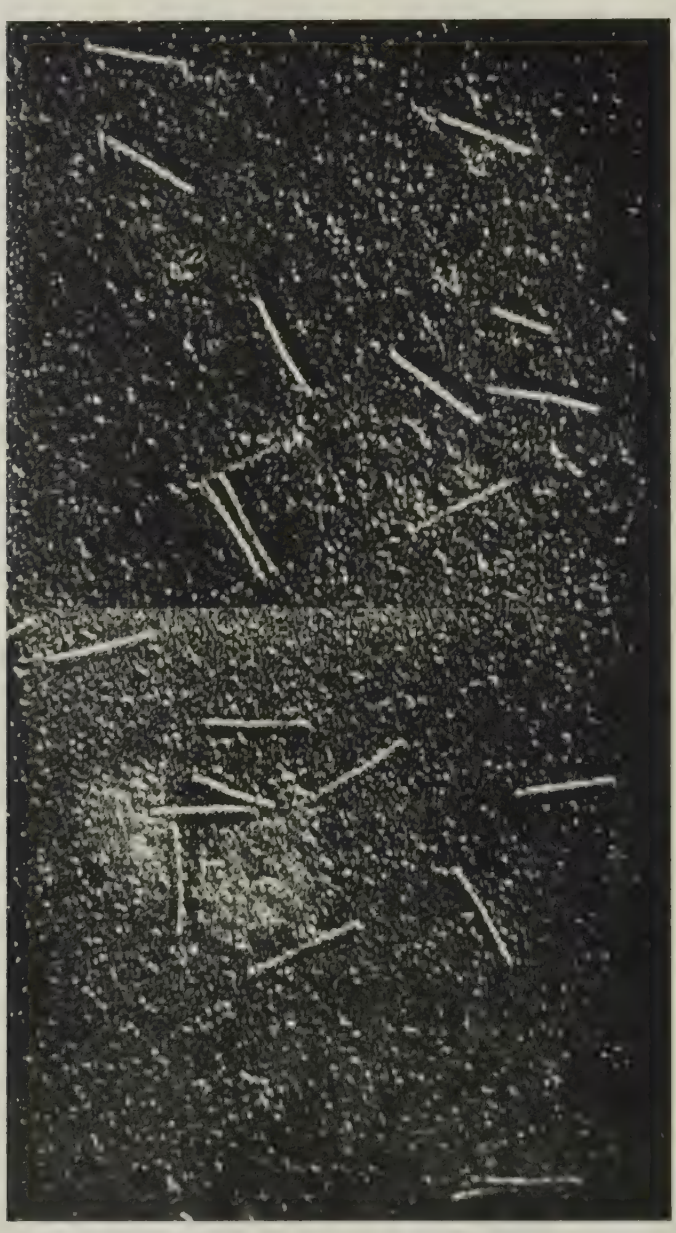

Fig. 4. Electron micrograph of cucumber virus 4 particles prepared from a diluted homogenat of infected leaves. Magnified by 50 000.

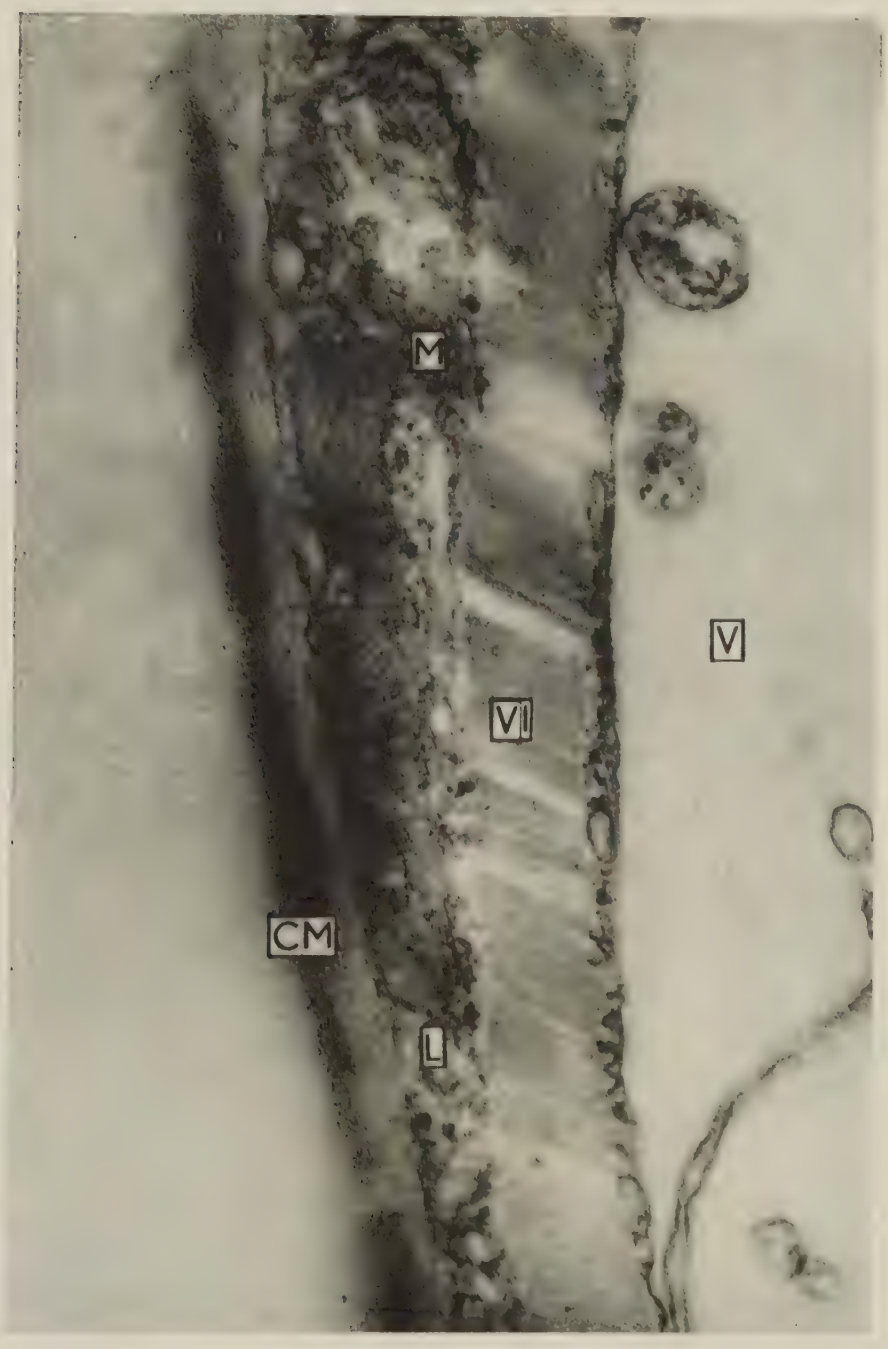

Fig. 5.

Figs. 5, 6, 7. Ultrathin sections of young cucumber leaves infected by cucumber virus 4. Inclusion composed of virus particles is enclosed in the cytoplasm. Fixation OsO_4 — buffer according to Buvat. Figs. 5, 6 direct magnification 22 500, total 50 600. Fig. 7 direct magnification 6000, total 20 000.

Fig. 6.

Fig. 7.

V — vacuole, VI — virus inclusion, M — mitochondria, CM — cell membrane, L — lysosome-like body, CH — atypical chloroplast with reduced lamellar system.

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

Fig. 1. Vegetative phase of growing point of apple flower bud. CS cover scales, CS 1 places of formation of further cover scales. On July 15, 1959. (Magn. × 210.)

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

Fig. 2. FF first flower primordium, SF second flower primordium. On Aug. 1, 1959. (Magn. × 200.)

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

Fig. 3. FF first flower primordium, SF second flower primordium. On Aug. 8, 1959. (Magn. × 200.)

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

Fig. 4. SP sepal primordium, SF second flower primordium. On Aug. 11, 1959. (Magn. × 215.)

Fig. 5. FF first flower, SF second flower, SP sepal primordium. On Aug. 15, 1959. (Magn. × 200.)

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

Fig. 6. SP sepal. On Aug. 18, 1959. (Magn. × 200.)

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

Fig. 7. PT petal primordium, SP sepal primordium, SP 1 sepal primordium of second flower. On Aug. 25,1959. (Magn. × 210.)

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

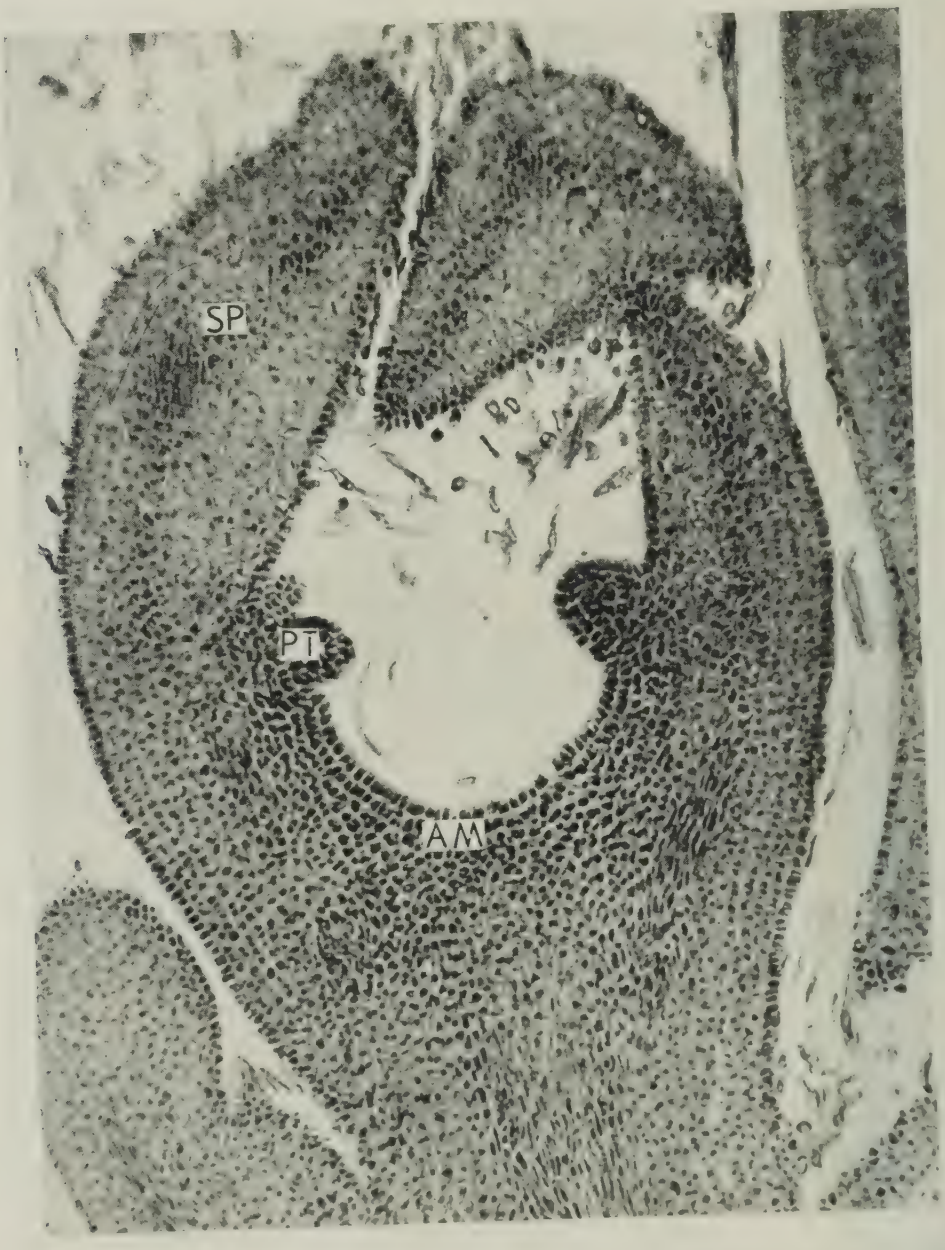

Fig. 8. SP sepal primordium, PT petal primordium, AM apical meristem. On Sept. 1, 1959. (Magn. × 185.)

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

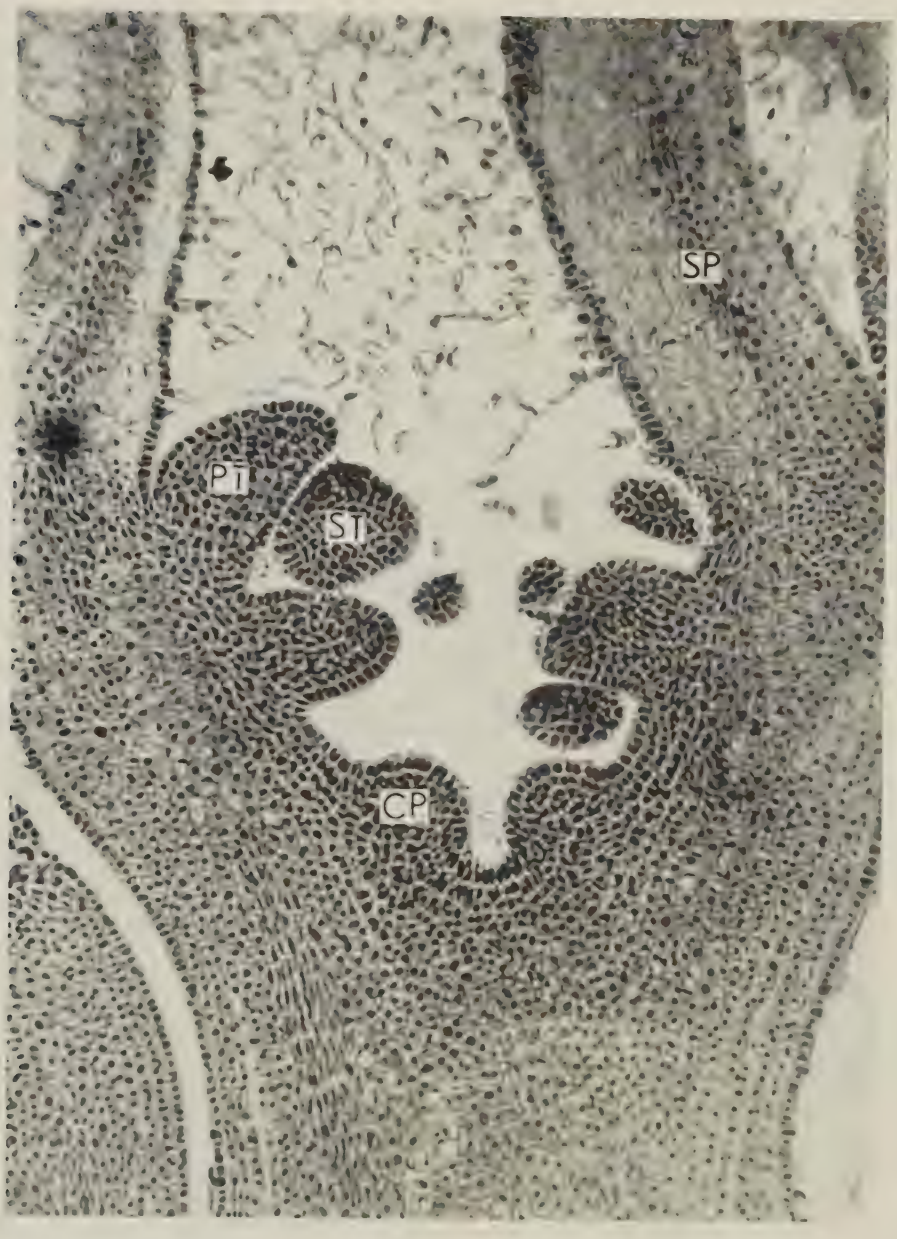

Fig. 9. SP sepal primordium, PT petal primordium, ST stamen primordium, CP carpel primordium. On Sept. 9, 1959. (Magn. × 190.)

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

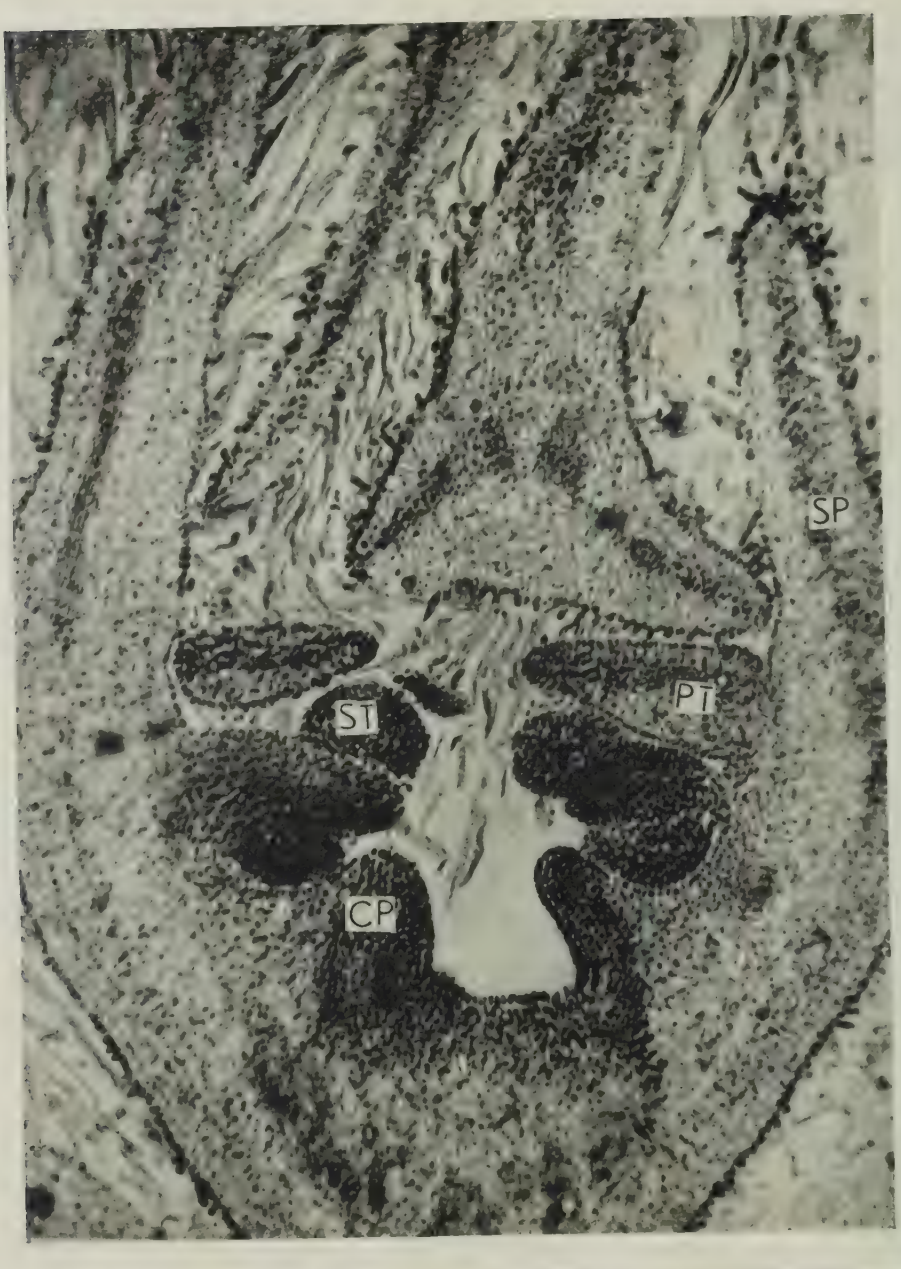

Fig. 10. SP sepal primordium, PT petal primordium, ST stamen primordium, CP carpel primordium. On Sept. 15, 1959. (Magn. × 130.)

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

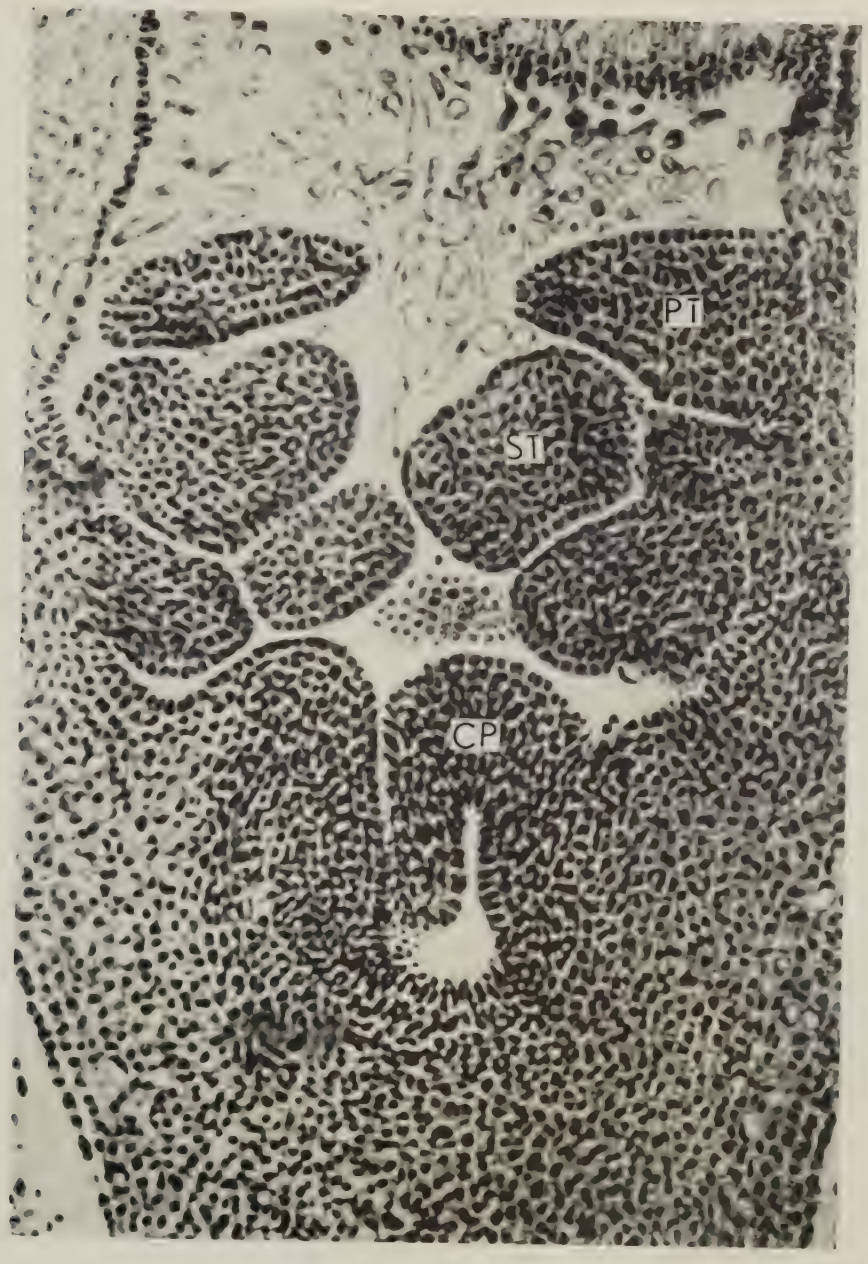

Fig. 11. PT petal primordium, ST stamen primordium, CP carpel primordium. On Nov. 11, 1959. (Magn. × 130.)

MICROPHOTOGRAPHY OF PHASES IN THE DEVELOPMENT

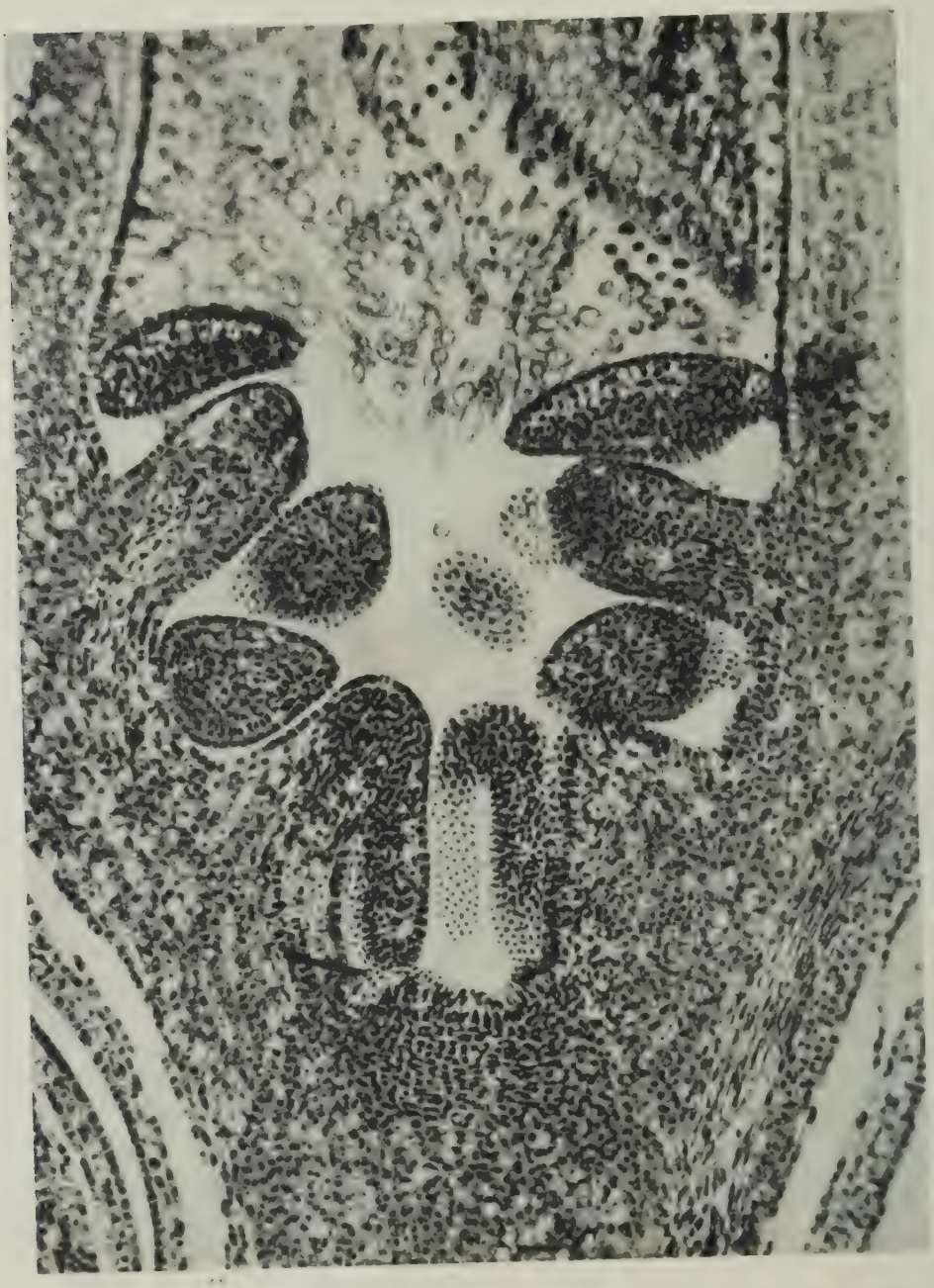

Fig. 12. On Jan. 4, 1960. (Magn. ~ 200.)

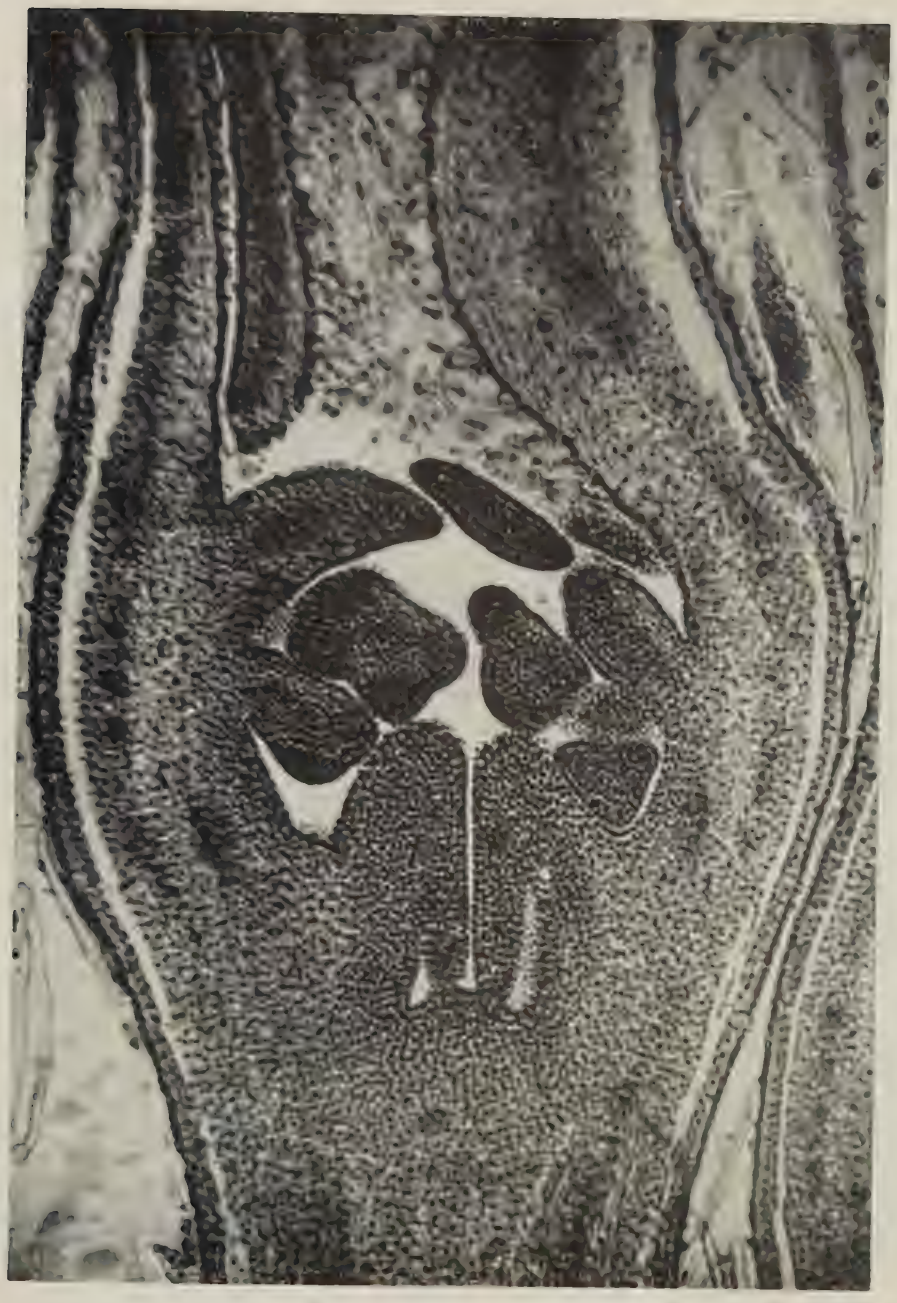

Fig. 13. On March 14, 1960. (Magn. × 100.)

Fig. 14. PT petal, ST stamen, CP carpel, O ovary. On Apr. 4, 1960. (Magn. × 100.)

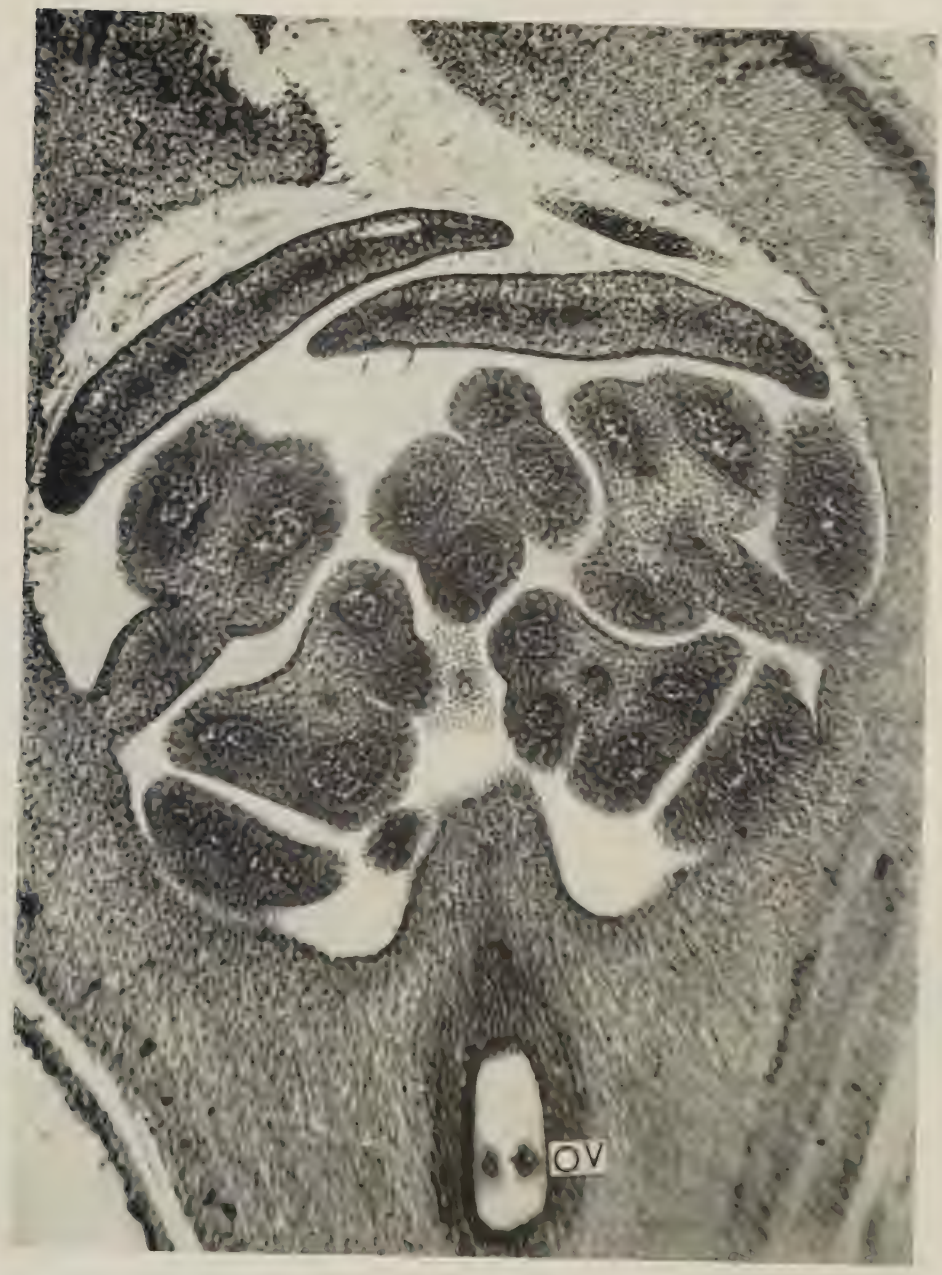

Fig. 15. OV ovule. On Apr. 4, 1960. (Magn. × 100.)

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Microphotography of Phases in the Development of Apple Flower Buds

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Mikrofotografie fází vývoje květních pupenů jabloně

Je uveden vývoj květních pupenů jabloní od jejich vzniku na branchyblastech až do doby krátce před vykvetením. Celé období vývoje je možné rozdělit do čtyř hlavních fází: I. fáze je vegetativní, v níž vznikají na vzrostném vrcholu primordia krycích šupin. II. fáze zahrnuje morfologickou diferenciaci vzrostného vrcholu, při níž se vytvářejí primordia květů. III. fáze zahrnuje vznik květních orgánů. IV. fáze je kvetení.

Summary

The development of flower buds of the apple from their formation on the brachyblasts until shortly before flowering is described. It is possible to divide the whole period of development into four main phases:

I, the vegetative phase in which primordia of scale leaves are being formed at the growing point. II, includes morphological differentiation of the growing point during which primordia of flowers are formed. III, covers the formation of flower organs. IV, is the flowering stage.

Material and Methods

Apex buds of brachyblasts of the Lord Lambourne apple variety were fixed in 70% ethanol, dehydrated in tertiary butanol according to Johansen (1940) and embedded in paraffin wax.

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Results

The preparations were cut into sections 7 μ thick and stained with Hansen's haematoxylin (Wolf 1940). In most cases, only the first flowers laid down in the bud are shown on the photographs.

During the first phase, primordia scale leaves detach themselves from the growing point (fig. 1). In the morphological differentiation phase (fig. 2), the growing point of the bud gives quite a different picture. The first flower primordium is formed and in a few days, primordia of the second flowers, usually 5 to 7 in number (fig. 3). Immediately after morphological differentiation, the third phase begins first with primordium of the first flower, followed by the laying down of further flowers. In this phase, sepal primordia are first formed (fig. 4), which gradually grow bigger (figs. 5 and 6). Towards the end of the second half of August, petal primordia are formed (fig. 7). In the meantime, the sepals grew considerably (fig. 8). Further flower organs, which are formed almost simultaneously, are stamen primordia and carpel primordia (fig. 9). From then on, the picture remains practically unchanged until the beginning of spring (figs. 10, 11, 12 and 13). Shortly before flowering, a vigorous growth of all flower parts takes place (figs. 14 and 15). The last phase is flowering.

Because of technical difficulties in the preparation of permanent microscopic preparations, the stages of development of the flowers shortly before flowering, are not given.

In single varieties grown under various geographical, climatic and soil conditions, and in different years, certain time differences exist in the development of the flower buds, especially with regard to the morphological differentiation of the growing point (Hořavka 1957). Given calendar dates of the beginning of single phases of the development of flower buds are therefore only valid for the given variety of apple, Lord Lambourne, during the period 1959—60 and the Experimental base of the Biological Institute at Střížovice near Trutnov and it is impossible to generalize them.

The development of apple flower buds has already been described several times and documented by illustrations (Bijhouwer 1924), but the preparation technique is too clumsy for more detailed study of this question and further fails to register numerous details which are only perceptible on thin microscopic sections.

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Микрофотография фаз развития цветочных почек яблони

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Автор исследовал развитие цветочных почек яблони, начиная с их образования на брахибластах и кончая стадией развития, предшествующей началу цветения. Все развитие можно разделить на четыре главных фазы:

1. вегетативная фаза, в которой в точке роста возникают закладки покровных чешуй,
2. фаза, включающая морфологическую дифференциацию точки роста, при которой возникают закладки цветков,
3. фаза образования цветочных органов,
4. фаза цветения.

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Влияние кининов на рост почек яблони и на включение P^{32}

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Vliv kininů na růst pupenů jabloně a na inkorporaci radiofosfátů P^{32}

Kinetin a 6-benzylaminopurin byly aplikovány u jabloně (*Malus silvestris*, Mill.) s cílem regulace růstu a metabolizmu pupenů. Jednorázová dávka 20 μg až 200 μg kininu překonala inhibiční účinek plodu na růst pupenů. Nižší dávky mají za následek sílení pupenů, vyšší dávky prorůstání pupenů ve větévky.

Pomocí radiofosfátu bylo zjištěno, že inhibiční vliv plodů se projevuje zejména v podstatném snížení inkorporace radiofosfátu do frakce volných nukleotidů hlavně v meristematických pletivech. Dále je podstatně snížena rychlost biosynthesy nukleoproteidů a fosfolipidů v pupenech plodící jabloně ve srovnání s pupeny jabloně, která je v daném roce bez plodů.

Kininy způsobují změnu metabolizmu pupenů sousedících s plody (jak meristémů tak i šupin) a inkorporace radiofosfátu je pak po sedmidenním působení velmi podobná inkorporaci v pupenech jabloně, která je bez plodů.

Synthetisovali jsme radioaktivní 6-benzylaminopurin-9-C^{14} a metodou radioautografie jsme zjistili, že se lokalisuje v blízkosti místa podání, a to převážně v primární kůře a floemu. K apikálnímu konci je transportován v malé míře, směrem k basálnímu konci výhonu ve větší míře (zčernání Rtg filmu ve vzdálenosti několika cm od místa podání). Po delší době byla z pokusného materiálu isolována další radioaktivní látka, která se od podaného 6-benzylaminopurinu liší svým Rf při papírové chromatografii. Zkoumáním této látky se zabýváme v další práci.

Резюме

1) 6-фурфуриламинопурин и 6-бензиламинопурин вызывают в низших дозах утолщение почек яблони а в более высоких дозах прорастание почек в побеги. 20—50 микрограмм 6-бензиламинопурина или 100—200 микрограмм кинетина устраняет подавляющее влияние плодов на рост почек замещения на плодовых веточках яблони. Почки разных видов и сортов

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плодовых деревьев утолщаются и растут под влиянием кининов в разной степени. Стимулирующее влияние кининов зависит также от сроков применения в течении вегетационного периода.

2) Изучением скорости включения радиофосфата P^{32} было найдено, что подавляющее влияние плодов на рост почек отражается в первую очередь на снижении скорости включения радиофосфата в свободные нуклеотиды, нуклеопротеиды и фосфолипиды особенно в верхушечных меристемах почек.

3) Кинины вызывают изменение метаболизма почек, которые расположены вблизи плодов а в том направлении, что уже на седьмой день после введения кининов в черешок листа стимулированной почки была найдена почти одинакова скорость включения радиофосфата как в стимулированных почках плодоносящей яблони, так и в контрольных почках не плодоносящей яблони.

4) Меченый 6-бензиламинопурин-9-C^{14} введенный в проводящие пути побега яблони концентрируется главным образом в коре и флоэме. В направлении к верхушке побега транспортируется в малой степени, в направлении к основанию побега в большей степени (потемнение на рентгеновой пленке на расстоянии нескольких см от места, в которое был введен раствор кинина). По истечении более продолжительного времени после подачи 6-бензиламинопурина-9-C^{14} было из тканей побега яблони изолировано новое радиоактивное вещество, которое отличается от введенного в побег кинина более низким Рф при бумажной хроматографии. Это вещество изучаем в дальнейшем.

Введение

В сороковых годах было найдено, что в кокосовом молоке находятся стимуляторы роста растительных тканей. Стронг со своими сотрудниками уже с 1950 г. начал работать над выделением этих веществ в чистом виде, но только в 1956 году им удалось изолировать действующее начало — 6-фурфуриламинопурин — из водной вытяжки дрожжей а также из дезоксирибонуклеиновой кислоты, полученной раньше из спермиев рыбы. Выделенное вещество наводит деление клеток растительных тканей и поэтому было названо — кинетином. Позже было синтезировано до 30 веществ сходного строения и действия с кинетином и поэтому получили групповое название — кинины.

Из дальнейших работ занимающихся действием кининов на растения мы упомянем лишь те из них, которые ближе относятся к решаемой проблеме. Так Бриан с сотрудниками (Brian et al, 1957) нашли, что кинетин действует на рост интернодиев гороха только в присутствии гетероауксина и тем отличается от действия гиббереллина. Гамприз ((Humpries, 1958) опрыскивал раствором кинетина молодые растения фасоли (*Phaseolus vulgaris* L.), в результате чего листья этого растения росли медленнее чем контрольные. Варианты опрыскиваемые гиббереллином отличались, наоборот, от контроля усиленным ростом площади листа.

Лона и Боччи (Lona, Bocchi, 1957) опрыскивали кинетином несколько видов однолетних растений, в результате чего наблюдали более интенсивное ветвление стебля. Виксон и Тиман (Wickson, Thiman, 1958) нашли, что у гороха (*Pisum sativum* L) проявляется антагонистическое действие кинетина и β-индолилуксусной кислоты при росте латеральных почек стебля. Ричмонд и Ланг (Richmond, Lang, 1957) сообщили, что кинетин замедляет процессы пожелтения листьев и распад белков в этих листьях. Мотес (Mothes,, 1960) подтвердил и объяснил это явление в недавно опубликованной работе о влиянии кинетина на включение глицина-C^{14} в листья табака (*Nicotiana tabacum*).

Чайлахян и Бутенко (1959) изучили влияние кинетина на закладку цветочных почек у периллы (*Perilla nankinensis* Lour).

Мы до сих пор не находим в литературе данных о влиянии кининов на деревья. При исследовании вопроса о влиянии плодов на рост и развитие почек замещения на плодушках яблони (Malus) мы нашли, что у периодически плодоносящих сортов плоды замедляют или ингибируют прежде всего процессы роста и деление клеток в почках, которые расположены вблизи плодов и что кинины «преодолевают торможение деления клеток» (Хвойка, 1960). Ингибирующее действие плодов на рост верхушечных почек брахибластов до сих пор не изучено. Из работ Лаквилла в то же время известно, что в семенах и плодах возникает несколько стимуляторов и ингибиторов роста (Luckwill, 1957). После ознакомления с работой Виксона и Тимана (Wickson, Thiman, 1958) нам сейчас известно, что кинетин может преодолеть тормозящее влияние гетероауксина, который однако, по видимому, у яблони не имеется (Luckwill,, 1956).

Стронг с сотрудниками впервые синтезировали кинетин, однако и они не получили чистого препарата (Strong, 1958). С тех пор многие интересуются получением кинетина и поэтому было испытано несколько способов синтетического получения кинетина. В нашей работе мы использовали метод, который впервые применил Булок с сотрудниками (Bullock et al., 1957). Мы синтетизировали 6-фурфуриламинопурин и его бензиланалог, 6-бензиламинопурин а также меченые препараты с радиоуглеродом C^{14} на 9-ом углероду пуринового кольца.

Методика и объекты

1) Опыты были проведены на 2—12-летних деревьях яблони, растущих (окулированных) на вегетативно размножаемых подвоях М IX и М I. У сорта Боскопский красавец красный мы вводили 6 бензиламинопурин в дозе 50 микрограмм в растворе в черешки листьев на побегах двух- и трехлетних деревьев. У сорта Онтарио было это вещество введено таким же образом на плодушках плодоносящих и не плодоносящих деревьев. 6-бензиламинопурин мы растворяли в редестилированной воде в концентрации 0,05 мг/мл (0,005%) всегда перед началом опыта. В качестве контроля мы использовали растворитель.

2) Методика скорости включения радиофосфата P^{32} в почки: Спустя 7 дней после введения 6-бензиламинопурина был в опытные деревья введен раствор KH_2PO_4, который содержал в расчете на одно дерево около 0,5 мС радиоизотопа P^{32} и около 50 мг общего фосфора. Раствор был введен в две-три скелетных ветви, из которых отбирались

после этого почки для анализа. Этот способ введения мы использовали с учетом результатов нашей прежней работы (Хвойка, Бабицкы, 1960). Меченый фосфат был введен около семи часов вечера и следующим утром были отобраны пробы радиоактивных почек для анализа. После взвешивания была из почек удалена вода при помощи лиофильной сушки. Перед анализом была каждая проба разделена на две части: на чешуи и меристематическую часть, в которых был проведен анализ отдельно. Количество проб и повторений было ограничено количеством пробирок в центрифуге с охлаждением, поскольку мы применили кислую экстракцию.

После гомогенизирования с кварцевым песком в 1% $HClO_4$ была отделена кислая вытяжка на центрифуге при 0—4 град. С и 8000 G в течение 15 минут. Экстракция была повторена дважды.

Свободные нуклеотиды были адсорбированы на карборафин на качалке при охлаждении в течение 10 минут, после чего был карборафин отделен на центрифуге и в надосадочной жидкости адсорбция повторена еще два раза.

Оставшаяся кислая вытяжка (после отделения свободных нуклеотидов) была доведена до объема 20 мл и использована для определения неорганического радиофосфата и общего радиофосфата. Неорганический фосфат был экстрагирован количественно смесью органических растворителей (изобутильалкоголь + бензен) по методике Мартина и Доты (Martin, Doty, 1949).

Оставшиеся органические фосфорные соединения (после отделения неорганического фосфора) были минерализованы на песчаной бане (H_2O_2 + $HClO_4$), после чего был определен общий фосфор по методу Мартина и Доты, как и выше. Из разницы между общим фосфором и неорганическим фосфором было вычислено количество радиоизотопа фосфора, включенное в фосфорные эфиры.

Свободные нуклеотиды были элюированы с карборафина 5 раз 25% этилалкоголем с 0,5% NH_4OH. (Около 10% не элюировалось.) Количество радиоизотопа P^{32} было определено после минерализации (в муфельной печи) и растворения в 1 Н серной кислоте в объеме 10 мл на счетчику 6 М для измерения жидкостей. Измерение всех остальных фракций и соединений было проведено одинаковым образом.

Сразу после отделения кислой фракции была из осадка в центрифужных пробирках удалена кислота с помощью повторных вытяжек этилалкоголем при 0—4 град. для того, чтобы при следующих вытяжках при комнатной температуре не отщеплялись пурины от нуклеиновой кислоты. Фосфолипиды экстрагировались в дальнейшем смесью этилалкоголя с эфиром в соотношении 3 : 1, потом 1 : 1 и наконец только эфиром. Каждая экстракция продолжалась несколько часов, поэтому удаление фосфолипидов продолжалось несколько дней. После того фосфолипиды сжигались в муфельной печи и определялась радиоактивность как и выше.

Этот способ фракционирования был использован раньше в работе Зайцевой, Белозерского, и Новожиловой (1959).

Из сухого остатка после удаления фосфолипидов был удален эфир и после прибавления 0,5 Н КОН был проведен гидролиз РНК в течение 18 часов при 37 град. Ц — этот способ оправдался раньше в работах Ванюшина и Белозерского (1959). РНК была отделена от ДНК после доведения концентрации водородных йонов до pH = 1 на центрифуге при 8000 G при 0—4 град. С в течение 15 минут. Остаток ДНК и белков был промыт 0,5% $HClO_4$.

3) Приговление ауторадиограммы побега с меченным кинином: В черешок листа однолетнего побега яблони был введен 6-бензиламинопурин-9-C^{14} (сорт Ренет Берлепш) и после истечения 6 дней был опытный побег разрезан на части в поперечном и в продольном направлении. После подсушки была изготовлена ауторадиограмма на пленке Ртг Агфа с недельной экспозицией.

4) Синтез пуриновых производных: Нерадиоактивные 6-фурфуриламино- и 6-бензиламинопурин были синтезированы по способу Булока (Bullock, 1957).

6-бензиламинопурин-9-C^{14} был синтезирован конденсацией 6-хлорпурина-9-C^{14} с бензиламином. 6-хлорпурин-9-C^{14} мы синтезировали путем приготовления гипоксантина-9-C^{14} по методу Монтгомеры) (Montgomery, 1958). Выход (продукт) конденсации бензиламина с 6-хлорпурином-9-C^{14} в метилцелосолве составляет 69%, а его точка таяния 223 град. Ц. Спектрум поглощения в ультрафиолетовой области и Рф при бумажной хроматографии соответствуют литературным данным. Удельная активность препарата была 1 микрокюри/мг.

Результаты и обсуждение

Физиологическое действие кининов

В 1959 году мы нашли (Хвойка, 1960), что кинетин стимулирует рост почек яблони, которые не превращаются в цветочные почки из-за отрицательного воздействия плодов на рост и развитие этих почек.

Позже мы нашли, что другой аналог кинетина — 6-бензиламинопурин — стимулирует рост почек плодовых деревьев в дозах 2—4 раза низших, чем кинетин. Кинетин стимулировал рост почек лучше всего в дозах 100—200 микрограмм, в то время как 6-бензиламинопурин в дозах близких 50 микрограммам. Фото №№ 1—5 изображают типичные случаи растущих стимулированных почек.

Действие кининов на рост почек яблони зависит прежде всего от состояния организма в разное время года. В наших опытах мы наблюдали стимулирующее действие от июня до октября. В первой половине вегетационного сезона кинины действовали больше всего на рост почек (июнь, июль). Почки стимулированные в сентябре и октябре месяце уже не прорастают в побеги а только увеличивают свой объем, утолщаются. В 1960 году мы стимулировали около 1000 почек, из которых около 60% проросло в более или менее длинные веточки. Контрольные почки не росли.

Во вторую очередь нужно учитывать сорт яблони, от которого также в большой степени зависит действие кининов. Этот вопрос не входит в рамки нашей работы, поэтому ограничимся лишь замечанием. Так например сорт Лорд Ламборн слабо реагирует на введенный кинин, в то время как сорт Боскопский красавец красный слабо реагирует на кинетин, но после 6-бензиламинопурина почки прорастают в веточки.

В настоящее время еще трудно объяснить, почему оба кинина не действуют в одинаковой степени и почему у разных сортов действуют по разному. Можно предполагать, что после объяснения механизма их действия на биохимические и физиологические процессы можно будет объяснить также отличия воздействия в отдельных случаях.

Пока можно с уверенностью сказать, что отличия в физико-химических качествах кининов должны сказываться в их различном действии на ростовые процессы. Так например 6-бензиламинопурин отличается лучшей растворимостью в воде по сравнении с кинетином и этим, кажется, можно до некоторой степени объяснить их различную эффективность.

Вопрос об эффективности действия кининов на рост почек зависит также от передвижения и локализирования этих веществ в растительных тканях. С целью определения концентрации 6-бензиламинопурина в отдельных тканях мы провели следующий опыт.

В черешок листа однолетнего побега яблони сорта Ренет Берлепш было введено 100 микрограмм 6-бензиламинопурина-9 C^{14}. После 6 дней был побег разрезан на части в поперечном и продольном направлениях и определена относительная концентрация кинина способом ауторадиографии.

На фото №№ 6,6а видим в наглядной форме, что из черешка листа в направлении к основанию побега расположен главный путь передвижения

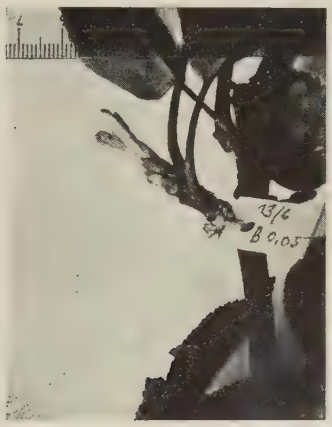

Фото № 1. Прорастание верхушечной почки брахибласта после введения 50 микрограмм 6-бензифминопурина 13/VI — 1960. Сорт Онтарио 9 лет.

Фото № 2. Почки брахибластов после введения 50 μг 6-бензиламинопурина 22/VI—1960 проросли в побеги. Сорт Боскопский красавец красный, 4 года.

Фото № 3. Стимулированные почки на однолетних побегах прорастают после введения 6-бензиламинопурина 19/VII-1960. Боскопский крас. 2 года.

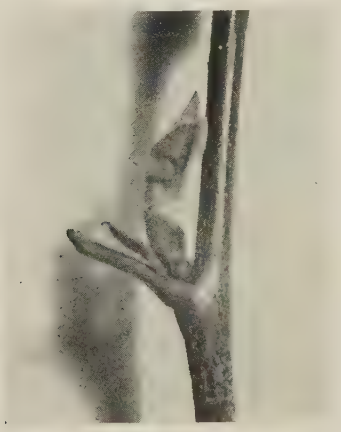

Фото № 4. После введения 6-бензиламинопурина в черешок одного листа у основания побега утолщились и почки в середине побега. Боскопский крас., 2 года.

6

6a

Фото № 6 и 6а — на светлом фоне сфотографированы разрезанные части побега, в который был введен 6-бензиламинопурин-9-C^{14} — на черном фоне ауторадиограмма тех же частей.

Фото № 5. Почки утолщаются после введения 50 μг 6-бензиламинопурина в сентябре 1960. Боскопский крас. 4 года.

меченого кинина. Его концентрация падает сильно в направлении к верхушке побега и более медленно в направлении к основанию побега.

Не без интереса то обстоятельство, что в области наибольшей концентрации кинина, под почкой, и возле почки, наблюдалось также наиболее интенсивное утолщение побегов. Побеги утолщались примерно одновременно с наиболее интенсивным ростом стимулированных почек, и это утолщение также зависит от времени (состояние организма), от химической структуры кинина и от сорта яблони.

В другом опыте было введено 100 микрограмм 6-бензиламинопурина-9-C^{14} в подобный побег, но не в черешок листа, а в декапитированную верхушку побега с помощью резиновой и стеклянной трубки, из которой раствор кинина мог свободно всасываться в проводящие пути ксилемы и флоэмы.

Результат ауторадиографии был сходный с выше изложенным. Меченый кинин концентрировался опять главным образом в коре и флоэме. Небольшая радиоактивность была также в ксилемной части, что можно отчасти объяснять за счет способа введения и приготовления препаратов для ауторадиографии.

Скорость включения радиоизотопа P^{32} в почки яблони

В 1959 году мы нашли, что скорость включения меченого фосфата в почки замещения на плодушках плодоносящей и неплодоносящей яблони не одинакова. Разница включения P^{32} начинает проявляться уже в начале июня, позже увеличивается и состоит в том, что скорость включения P^{32} в свободные нуклеотиды, нуклеиновые кислоты и фосфолипиды в почках плодоносящей яблони гораздо ниже, чем в почках яблони, из которой были удалены цветы, или молодые плодики.

В таблице № 1 приведены данные, которые иллюстрируют скорость включения меченого фосфата в почки брахибластов сорта Онтарио. Радиофосфат был введен 4-го августа в 19,00 часов. Радиоактивные почки были срезаны 5-го августа в 7,00 часов, на седьмой день после введения 6-бензиламинопурина в почки брахибластов плодоносящей яблони.

У яблони без плодов включилось около 6% радиофосфата в нуклеопротеиды меристематической части почек, в то время как у плодоносящей яблони в два раза меньше. Сходную разницу наблюдаем также у фосфолипидов. Самая большая разница у свободных нуклеотидов, большую часть из которых составляют соединения содержащие аденин (найдено хроматографией на Дауэкс-1).

Большого внимания заслуживает тот факт, что каталитическая доза 6-бензиламинопурина однозначно стимулирует синтетические процессы в меристемах и листовых чешуях почек, в результате чего уже после 7 дней от введения кинина скорость включения радиофосфата в почки плодоносящей яблони восстановилась до уровня не плодоносящей яблони. В связи с методическими затруднениями мы не смогли провести большое количество анализов, из которых можно было бы вычислить статистическую достоверность данных. Важно, однако, то, что скорость включения радиофосфата в свободные нуклеотиды, нуклеопротеиды и фосфолипиды хорошо совпа-

Таблица № 1

Скорость включения P^{32} в чешуи и меристемы почек яблони

Название соединений	Радиоактивность чешуй		Радиоактивность меристем	
	Имп./мин.	%	Имп./мин.	%
Контрольные почки не плодоносящей яблони				
Сумма	17.161	100,00	7.532	
Неогранический фосфор	8.960	52,21	2.590	34,4
Фосфолипиды	1.165	6,78	490	6,5
Свободные нуклеотиды	243	1,41	299	3,96
Фосфорные эфиры	5.990	34,92	3.655	48,54
Нуклеопр теиды	803	4,68	498	6,60
Сумма	17.161	100,00	7.532	100,00
Органический фосфор		47,79		65,60
Почки плодоносящей яблони 7 дней после введения 6-бензиламинопурина (50 микрограмм) в черешок листа				
Неогранический фосфор	20.076	44,16	5.453	34,55
Фосфолипиды	3.185	7,00	1.182	7,49
Свободные нуклеотиды	655	1,43	1.160	7,34
Фосфорные эфиры	19.080	41,96	6.825	43,25
Нуклеопротеиды	2.481	5.45	1.163	7,37
Сумма	45.477	100,00	15.483	100,00
Органический фосфор		55,84		65,45
Контрольные почки плодоносящей яблони				
Неорганический фосфор	17.192	38,50	12.313	43,64
Фосфолипиды	2.393	5,36	877	3,10
Свободные нуклеотиды	310	0,69	292	1,03
Фосфорные эфиры	23.175	51,90	13.975	49,53
Нуклеопротеиды	1.586	3,55	763	2,70
Сумма	44.656	100,00	28.220	100,00
Органический фосфор		61,5		56,36

дает с интенсивностью роста почек; а рост почек так или иначе должен быть результатом усиления синтеза не только белков, как это имело место в опытах Мотеса (Мотнеs, 1960), но также и нуклеиновые кислоты и фосфолипиды необходимы для образования новых клеток, которое наблюдается как результат стимулирующего действия кининов.

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The Effect of Kinins on the Growth of Apple-Tree Buds and on Incorporation of Radioactive Phosphate

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Kinetin and 6-benzylaminopurine were applied to apple-trees *(Malus domestica)* with the aim of regulating growth and metabolism of buds. A single dose of 20—200 μg. kinin counteracted the inhibitory effect of the fruit on bud growth. Lower doses resulted in thickening of buds, higher doses in bursting of buds to form twigs.

It was found with the aid of radioactive phosphate that the inhibitory effect of fruits is reflected particularly in a considerable decrease of incorporation of radioactive phosphate into the fraction of free nucleotides, especially in meristematic tissues. Furthermore the rate of synthesis of nucleoproteins and phospholipids is considerably decreased in the buds of a fruit-bearing apple-tree as compared with those of one which is barren in the given year.

The kinins bring about a change in the metabolism of buds adjacent to fruits (both of their meristem and scale integuments) and the incorporation of radioactive phosphate resembles after a seven-day exposure very much that in the buds of a barren apple-tree.

Radioactive 6-benzylaminopurine-9-^{14}C was synthesized here and autoradiography revealed that it is localized in the vicinity of the point of application, predominantly in the bark and in the phloem. It is little transported toward the apical end, somewhat more toward the basal end of the shoot (an X-ray film darkens several cm. away from the point of application). After an extended period of time another radioactive substance was isolated from the experimental material, the substance differring from 6-benzylaminopurine in its R_F value during paper chromatography. The substance is being further investigated.

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Disinfection of Kernels and its Influence on the Growth and Photosynthesis of Maize Seedlings

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Desinfekce obilek a její vliv na růst a fotosynthesu klíčních rostlin kukuřice

Při pěstování rostlin kukuřice ve sterilních kulturách je třeba obilky desinfikovat, aby se zničily zárodky mikroorganismů. To snižuje jejich klíčivost, zpozdí růst a tvorbu chlorofylu během prvních několika týdnů vývoje klíčních rostlinek. Nejde o specifické inhibice, nýbrž o vývojové opoždění neprojevující se ani v poměru dlouživého růstu a hromadění sušiny. Opoždění je největší při použití roztoku sublimátu nebo ethanolu. Vhodnější je desinfekce roztokem chloraminu. Změny vyvolané tříhodinovým namáčením jsou vyrovnány během cca 3 týdnů, běžně užívaná sedmihodinová desinfekce vyvolává kromě silného snížení klíčivosti (téměř o 50 %) déle trvající, avšak nezásadní změny. Chloramin neovlivnil tvorbu pohlavních orgánů, intensita fotosynthesy byla úměrná množství chlorofylu. Desinfekce chloraminem lze tedy užít, aniž by se rostliny podstatně lišily od těch, které byly vypěstovány ze suchých obilek.

Summary

Soaking of maize kernels in disinfectants reduces their germination capacity, and delays growth and chlorophyll formation during the first few weeks of the development of seedlings. These effects represent no specific inhibition, but rather a delay in development which is not even evinced in the ratio of elongation growth to the accumulation of dry matter. Delay is greatest when $HgCl_2$ solution or ethanol is used. Chloramine solution is suitable for disinfection purposes: changes produced by three hours' soaking in this solution are equalised in the course of about three weeks, while the usual seven-hour period of disinfection causes, in addition to pronounced reduction in germina-

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tion capacity (by nearly 50%), changes of longer duration, which are not, however, of a fundamental nature. Chloramine did not affect the formation of sex organs; the intensity of photosynthesis was proportionate to the amount of chlorophyll.

Introduction

When establishing sterile plant cultures it is necessary to disinfect the seeds before germination. The influence of disinfectant agents on plants has not been much investigated and published results refer in the most part to germination (Bruttini 1895, Sigmund 1896). Among the disinfectant agents most frequently employed in plant tissue culture technique are compounds containing active chlorine. Some authors (Sigmund 1896, Morse 1935), however, mention that some halogen elements and their compounds have an unfavourable effect on plant germination.

In view of the fact that in our experiments with sterile cultures of isolated tips of maize plants disinfection of kernels was carried out mainly by means of chloramine, it was necessary to find out whether the growth, development and metabolism of the cultivated plants suffer through the action of the disinfectant agent. The fundamental metabolic process was chosen for investigation, i.e. photosynthetic assimilation and one of its basic factors, chlorophyll content. For the purpose of comparison with the action of chloramine, disinfection was also carried out with $HgCl_2$ solution and ethanol.

Methods and Results

Cultivation of the plants

Maize (*Zea mays* L.) var. Stupická raná Elite was used in all experiments. In each experiment and in each variant there were 100 kernels from the central part of the cob; their average weight was 35·5 g. They were always soaked in 100 ml. of water or solution. The following preparations were used:

a) redistilled water from a glass apparatus (pH 6·3) or tap water (pH 6·7) and redistilled and tap water with pH 8·0 (adjusted with KOH solution);

b) 0·1% aqueous solution of $HgCl_2$;

c) ethanol 99% denaturated with 1% of benzine;

d) chloramine solution (Natrium benzensulphochloramidicum) — 10 g. of the substance containing 25% of active chlorine dissolved in 90 ml. of redistilled water.

Period of soaking 3 or 7 hours, with ethanol 30 min. or 3 hours.

Dry kernels were used as controls.

Following removal from the disinfectant agent the kernels were thoroughly washed with redistilled water; when disinfecting with mercuric chloride the kernels were first washed with Lugol's solution and then with water.

In view of the fact that non-disinfected kernels were used as controls, the plants could not be cultivated in vitro. They were therefore grown in boxes with garden soil in the greenhouse at an average temperature of 20° C. The experiments were carried out from the end of October to the end of March and no additional illumination was provided.

Germination

Germination was followed in the boxes with soil and, for comparison, in Petri dishes with 1% agar substrate; there were two to six repetitions in all. There were no essential differences between the two methods, and therefore a general average of the results is given in Table 1.

Table 1. The Effect of Soaking Maize Kernels in Disinfectant Solutions on their Germination Capacity

Method of treatment	pH of enviroment	Period of soaking in hours	Average % germination capacity
Dry kernels	—	—	93·3
H_2O redist.	6·3	3·0	91·5
H_2O redist.	6·3	7·0	93·2
H_2O redist.**	8·0	3·0	95·7
	8·0	7·0	97·5
Tap water	6·7	3·0	96·0
0·1% aq. sol. $HgCl_2$	—	3·0	97·0
Ethanol 99%	—	0·5	78·0
Chloramine solution*	8·0	3·0	85·5
	8·0	7·0	51·4

* At the end of soaking the solution is yellow.
** At the end of soaking the kernels are more yellow than at pH 6·3.

The kernels started to germinate after 4 to 5 days; the start of germination was delayed for 3 to 4 days by soaking in chloramine solution and ethanol, and for 2 days by soaking in mercuric chloride solution. The greatest reduction in the percentage of germinating capacity was observed with the seven-hour soaking in chloramine solution.

When the kernels are soaked in chloramine solution, the latter takes on a yellow colour. This is evidently not due to carotenoids, because it was not possible to shake out the pigment into methanol, acetone or ether. According to the colour reactions observed in visible and ultraviolet radiation, the compounds extracted belong to the flavonoids.

Observation of the formation of sex organs

Three to four days after the start of germination the seedlings were transplanted and divided into three groups:

1. Seedlings left with their kernels (control);
2. Endosperm isolated from seedlings;
3. Only an isolated part of the main stalk with the shoot apex retained (Petrů and Řetovský 1957).

There were 300 plants in each group: 100 plants from kernels soaked in chloramine solution, 100 plants from kernels soaked in water and 100 plants from dry kernels. The plants in the first and second groups showed almost uniform development and growth. Plants in the last group were retarded in growth and development; the third stage in the development of the tassel (Andrejenko, Kuperman 1959) started about 4 to 5 weeks later than in the controls. As compared with plants in groups 1 and 2, those in group 3 were

smaller, weaker and the tassel was considerably simplified (unbranched or only slightly branched); in some plants the tassel was completely sterile or there were indications of transformation of male flowers into female or well-developed female flowers were found alongside the male.

No noticeable differences were observed as regards the formation of the sex organs between plants from differently treated kernels.

Growth of the plants and chlorophyll content

Plants aged 10 to 32 days, which had 3 to 4 (occasionally 2 or 5) developed leaves, were used for the analyses. In view of the great differences in the lengths and colouring of the epicotyl, only top parts of plants cut off in all cases 1·5 cm. below the point of insertions of the lamina of the 1st leaf were used for analysis. Their maximum length was measured to the nearest millimetre; the fresh weight was determined to the nearest 10 mg.; their chlorophyl (a + b) content was then determined. A percentage of dry matter in the fresh weight of the plant top parts was determined in similar plants (by drying at 105° C to constant weight).

Plant pigments were extracted with acetone (water-free and 85%) from fresh material ground with sand and a trace of $MgCO_3$. After filtration through a dense glass sinter No. S 4, and after suitable dilution, extinction of the extract was measured on a Pulfrich photometer with the ELPHO objective attachment (Zeiss, Jena, Germany) using S 64 filter and 1 cm. long cuvettes. The amounts of chlorophylls (a + b) were calculated from calibration curves according to Dr. E. Wiedemann's (Basel, Switzerland) preparation for the ratio of chlorophylls a/b = 3/1.

Individual experiments with differently treated kernels were set up in the greenhouse during the winter from October to March, i.e. under variable climatic conditions, particularly as regards illumination. This was evinced in the dissimilar growth rates of plants in the different experiments; the character of the influence, which will be dealt with later, was uniform and therefore only the results of some typical experiments are given.

Table 2. Fresh Weight and Amount of Chlorophyll (a + b) in Plants Grown from Kernels Treated with Various Disinfectants. (Experiment 1/1-2-60. Average values from 6 plants.)

Pre-sowing treatment of kernels	Fresh weight in mg./plant		Chlorophyll (a + b) in mg./plant		"Coefficient of increase"*	
	after 12 days	after 26 days	after 12 days	after 26 days	fresh weight	chloro-phyll
0·1% aq. sol. $HgCl_2$; 3 hrs	146	833	0·192	0·831	1·70	1·32
Chloramine; 3 hrs	247	1491	0·235	1·660	1·80	2·14
Ethanol: 30 min.	181	1190	0·215	1·253	1·97	1·77
3 hrs	173	353	0·191	0·564	0·61	0·90
Tap water; 3 hrs	266	1020	0·267	1·220	1·14	1·39
H_2O redist.; 3 hrs (control)	355	1190	0·436	1·436	1·00	1·00

* "Coefficient of increase" states how many times greater the increase of the values followed was in the experimental variants than for the controls.

Experiment 1/1-2-60 (see Table 2) showed that chloramine is the most suitable of the disinfectant agents used because a three-hour soaking of the kernels caused the smallest retardation of growth and of chlorophyll formation during the first phase of the experiment (up to 12 days) and during the second phase of the experiment plants of this variant even outstripped the controls in the factors mentioned. The large initial inhibition caused by

sublimate was fairly rapidly overcome as regards growth, but less as regards chlorophyll formation (see "coefficients of increase" in Table 1). When kernels were soaked for 30 min. in ethanol the unfavourable initial effect was on the whole overcome, while three-hour action by ethanol clearly caused permanent damage to the plants' growth with an excessive accumulation of chlorophyll. The unfavourable effect of tap water in this experiment may have been due to temporarily stronger chlorisation; in another experiment using 4 types of water (tap water from two different water pipes, redistilled and well water) no statistically significant differences in either growth or chlorophyll content were obtained. Adjustment of the pH of the waters from between 6·3 and 6·7 to pH 8·0 evoked slower increase of length and fresh weight of the plants, while the amount of chlorophyll was proportionate to growth.

A somewhat higher chlorophyll content and greater amount of organic matter than in the controls was found in plants grown from kernels that had been soaked for three hours in chloramine solution in another experiment, too (see Table 3) — the differences were not, however, significant. Table 3 also gives the converted dry weight values (fresh weight determined x % dry weight from parallel plants): in plants of approximately the same development, differences in the form of the relation obtained when chlorophyll content is related to fresh weight or to dry weight are insignificant.

Table 3. Fresh Weight Calculated to Dry Weight and Amount of Chlorophylls (a + b) in Plants Grown from Kernels Soaked before Sowing in Water or for Three Hours in Chloramine Solution. (Experiment 4/1-60. Plants 13 days old. Average values from 12 plants.)

Method of treatment of kernels	Chlorophyll (a + b) in mg./plant	Fresh weight in mg./plant	Converted to dry wt. mg./plant	Coefficients	
				chloroph. in mg. / fresh weight in gr.	chloroph. in mg. / dry weight in mg. × 10^2
Chloramine; 3 hrs	0·548	458	45·1	1·19	1·21
Dry kernels	0·596	476	46·3	1·25	1·28
Tap water; 3 hrs	0·562	494	46·9	1·14	1·20
H_2O redist.; 3 hrs	0·433	427	40·9	1·01	1·06

In contrast to the three-hour treatment, seven-hour soaking of kernels in chloramine solution retarded growth in addition to considerable limitation of germination capacity. It is evident from Fig. 1 (further experiments gave similar results) that plants from chloramine-treated kernels are delayed in their development by only 2 to 4 days. When sampling is carried out on the same day, experimental plants are smaller than the controls, they have fewer leaves, lower fresh weight, but the amount of chlorophyll is proportionate to growth; there is therefore no question of any specific inhibition of chlorophyll formation.

Fig. 2 (from the same experiment) shows that treatment with chloramine did not even influence the ratio of fresh weight and dry weight of the plants. Only a retardation of growth of plants from kernels soaked in chloramine solution is noticeable. The linear relation between fresh and dry weights for

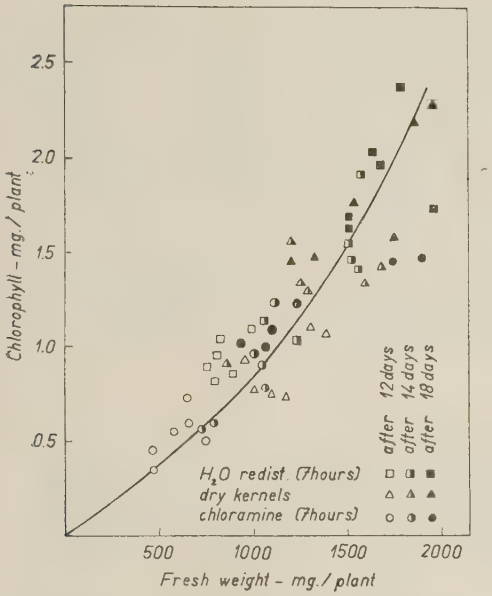

Fig. 1. Fresh Weight and Chlorophyll (a + + b) Content in 12, 14 and 18 day-old Plants Grown from Variously Treated Kernels (Experiment 3/1-3-60).

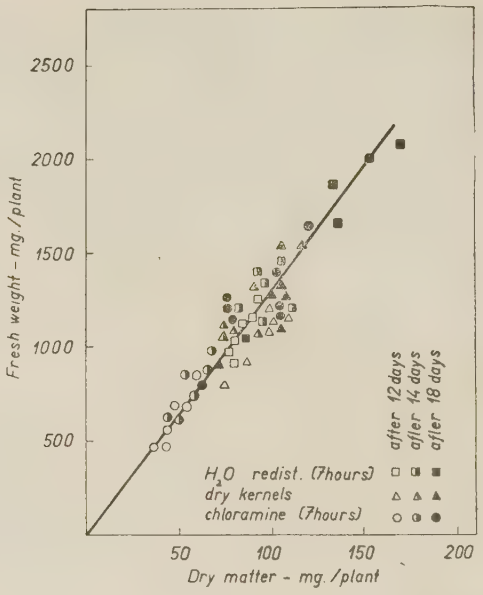

Fig. 2. Fresh and Dry Weights of 12, 14 and 18 day-old Plants Grown from Variously Treated Kernels (Experiment 3/1-3-60).

a given period of time also serves to demonstrate the justification of comparing chlorophyll content directly with the fresh weights of plants (as in Fig. 1).

The delaying of the growth of plants from chloramine treated kernels without affecting their elongation growth is also confirmed in Fig. 3 from another experiment. The relation between the lengths of the plants and their fresh weight is uniform in its character in all variants of the experiment.

Photosynthesis

The intensity of photosynthetic assimilation was determined using apparatus set up by Šesták and Šetlík, a detailed description of which will be published elsewhere. Disks of a total area of 25 cm.² are cut out from leaves of the experimental material and placed in a

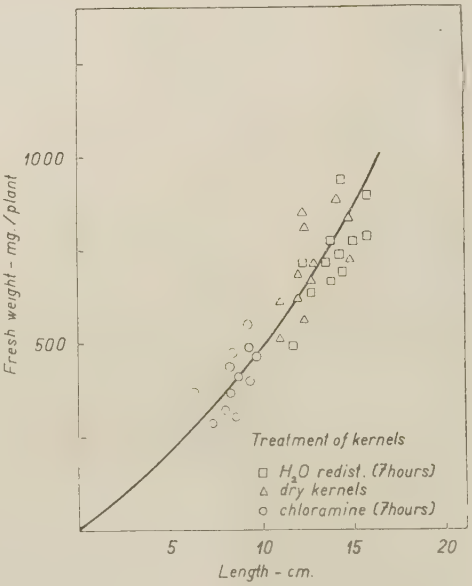

Fig. 3. Lengths of Top Parts of Experimental Plants and Their Fresh Weights.

chamber of plexiglass into a plate of spongy polyurethane saturated with water (see Bartoš, Kubín, Šetlík 1960). Illumination is provided by a Tungsram Kino E 40 707—1000 W bulb, light intensity being about 25,000 lx at the disk level. Air containing cca 1·0—1·2 mg. CO_2/l. circulates around the discs in a closed circuit. Differences in CO_2 concentration are detected by objective colorimetry from changes in pH of bicarbonate solution according to Kauko (1934), using Cresol Red as indicator. CO_2 loss is equalised in the closed circuit by addition of bicarbonate solution to H_2SO_4 by means of a microburette, with resultant liberation of stechiometric amounts of CO_2. The increase in weight of illuminated disks was determined by the method of Bartoš, Kubín, Šetlík (1960).The average temperature in the exposure chamber was +23° C, the period of light exposure three hours.

Plants used in the experiments were grown from kernels sown on January 15, 1960 (60 to 76 days old at the time of experiments) and February 9, 1960 (80—85 days old at the time of experiments). The kernels were soaked in water of various pH or in chloramine solution for 3 to 7 hours, or the kernels were sown dry. An average sample from two plants, 3 to 4 leaves from each plant (consecutive leaves within the range of the second to sixth fully developed leaves), was taken for one experiment. Photosynthetic activity was compared with chlorophyll (a + b) content in parallel disks.

Soaking in water and differences in the pH of water used did not influence the photosynthetic relation dependence under examination. Photosynthetic intensity depended only on the amount of chlorophyll and not on the method in which the kernels were treated before sowing; thus chloramine has no specific effect on photosynthetic activity and can only act through its influence on the formation of chlorophyll.

Discussion

The experiments were carried out in the greenhouse during the winter period and therefore under conditions of reduced light intensity. The temperature in the greenhouse was on the average 3° C lower than that considered to be the optimal for the growth of maize plants (Went 1957), but it is evident from the results obtained that the amount of chlorophyll and dry weight regularly correspond to Sprague and Shive's (1929) data for plants cultivated in the greenhouse.

In agreement with the somewhat scanty published results, it was observed that when using disinfectant agents containing active chlorine there is a reduction in the germination capacity of the kernels (proportionate to the length of soaking) and at the same time a higher statistical variability of maize seedlings. Irregularity in growth can be explained as due to differences in the degree of penetration of the solution through the pericarp into the kernels. In the course of three weeks the plants overcame the initial delay in growth and chlorophyll formation caused by three-hour soaking in chloramine solution. Although the seven-hour soaking of the kernels in chloramine—this period is essential for the destruction of microorganisms—gave greater differences as compared with the controls, not even in this case was there any question of specific inhibition of growth, chlorophyll formation or photosynthesis. Photosynthetic activity was proportionate to the amount of chlorophyll per unit of leaf area. In spite of the fact that chloramine strongly reduces germination capacity, it is found not to have any fundamentally unfavourable effect on the growth of seedlings. This cannot be said of the

effect of ethanol. This substance does not in itself guarantee good disinfection and any more prolonged action by it causes kernels to harden, while enzymatic activity is disturbed. $HgCl_2$ is a good disinfection agent, but the kernels must be washed in Lugol solution after soaking in order to remove all traces of mercury. This washing may explain the fact that, as opposed to SIGMUND (1896), no reduction in the germination capacity of kernels treated with $HgCl_2$ was found by the present authors. The effect on growth and on chlorophyll formation is greater after treatment with $HgCl_2$ than after chloramine, particularly in the first two weeks following the germination of the plants.

Acknowledgement:

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Дезинфекция зерен и ее влияние на рост и фотосинтез молодых растений кукурузы.

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При разведении растений кукурузы в стерильных культурах необходимо зерна предварительно дезинфицировать. Это, однако, влечет за собой снижение всхожести, запаздывание в развитии и в образовании хлорофилла в течение первых недель развития растений. В данном случае не имеет место специфическая ингибиция, а лишь запаздывание в развитии, которое даже не проявляется в нарушении соотношения между ростом в длину и накоплением сухого вещества. Наибольшее запаздывание наблюдалось в случае применения раствора сублимата или этанола. Более подходящим для дезинфекции является раствор хлорамина: изменения, вызванные трехчасовой обработкой, выравниваются примерно через три недели. Обычно применяемая семичасовая дезинфекция влечет за собой, кроме сильного (почти на 50 %) снижения всхожести, более продолжительные, но несущественные изменения. Хлорамин не влиял на образование половых органов, интенсивность фотосинтеза была пропорциональна количеству хлорофилла. Поэтому при дезинфекции с помощью раствора хлорамина полученные растения не отличаются существенно от тех, которые выросли из сухих зерен.

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Abnormities in the Inflorescence of *Triticum aestivum* E. after a Photoperiodic Treatment at Different Stages of Ontogeny

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Abnormity v květenství *Triticum aestivum* E. po fotoperiodickém zásahu v různou dobu ontogenese

Metodikou fotoperiodických pokusů a analys vzrostných vrcholů jsme zjistili závislost typu výsledné morfologické abnormity na vývojovém stupni vzrostného vrcholu před fotoperiodickým zásahem. Abnormálně velký počet klásků vznikal po zásahu u rostlin se zcela vegetativním vzrostným vrcholem. K větvení klasu došlo nejvíce po zásahu v době prodlužování vzrostného vrcholu. Abnormální vývin podpůrných listenů odpovídal zásahu mezi zakládáním klásků a zakládáním kvítků v klásceích. Čím dřívější byl tento zásah, tím úplněji byly podpůrné listeny vyvinuty. První dvě odchylky představují nadpočetný růst osních článků v květenství, vývin podpůrného listenu je známkou posunutí korelace mezi růstem listenu a generativním vývojem jeho úžlabního klásku. U poslední odchylky, řídkého klasu, která vzniká po zásahu v době zakládání tyčinek, již nedochází k takovému narušení vztahu mezi růstem a vývojem. Při fotoperiodickém zásahu dochází k indukci abnormální morfogenese, která pak může probíhat i po ukončení zásahu.

Summary

The technique of photoperiodic experiments and analysis of shoot apexes was used to determine the relationship of the type of the resulting morphological abnormity and the developmental stage of the shoot apex prior to the photoperiodic treatment. An abnormally high number of spikelets was formed when plants with a fully vegetative shoot apex were treated. Branching of the spike took place mostly after the treatment at the time of elonga-

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tion of the shoot apex. An abnormal development of bracts was induced when treated between the initiation of spikelets and the initiation of spike florets. The earlier this treatment was, the more fully the bracts were developed. The first two abnormities represent an excessive growth of internodes in the inflorescence, the development of a bract reflects a shift in the correlation between the growth of the bract and the generative development of its axillary spikelet. The last abnormity to be mentioned is a spike with elongated internodes which is formed subsequent to treatment at the time of initiation of stamina; at that time no considerable interference with the relationship between growth and development takes place. Photoperiodic treatment results in the induction of abnormal morphogenesis which may then take place even after termination of the treatment.

Introduction

It was shown in a previous paper (Blum and Kříž 1956) that a certain relationship exists between the abnormal formation of inflorescence following photoperiodic treatment and the ontogenetic stage of the plant at the time of this treatment. Observations of abnormities resembling those obtained here are known from descriptions in older teratological literature (Penzig 1921—22). Their formation brought about by an inhibiting photoperiodic régime has been described in numerous papers, many of them dealing with cereal spikes. Older references on the problem can be found in Fedorov and Batygin (1953). Of more recent papers dealing with the problem the following should be mentioned: Mathon (1954, 1955), Lekchinskaya and Viontsek (1955), Kuperman and co-workers (1955), Kuperman (1956), Wislocka (1955, 1959), Muschik (1957) and others.

In spite of the fact that none of these papers treated the problem of the relationship between the time of photoperiodic treatment and the resulting morphogenesis systematically, some of them provide an answer to some of the problems. Klebs (1918) found that abnormities occur only after a certain quite definite duration of photoperiodic induction. A similar time relationship was found for wheat by Garner and Allard (1925). A more detailed treatment and differentiation of the abnormities according to the time of photoperiodic treatment can be found in the work of Novikov (1953) where rye was used. A definite time relationship between the time of treatment of an inhibiting photoperiodic régime and the character of the abnormity was found by Leisle (1950, 1956) for a number of species and by Rylska (1960) for Perila. The problem of morphogenetic influence of photoperiodic treatment applied at different stages of ontogeny with respect to the developmental phase of the shoot apex prior to treatment in wheat was dealt with by Alleweldt (1958) and Du-Zho-Fu (1958a, b). Kuperman (1948) studied this relationship in an indirect way, using the technique of successive sowings and analyzing the development of shoot apexes. On the basis of her work she expressed the view that a delay during a certain stage in the development of the shoot apex results in the formation of a greater number of metameric organs, particularly of those which are being formed during the period in question on the shoot apex. Similar results were also obtained by Rostovtseva (1951) and Rzhanova (1951) with a number of grasses. The results of Resende (1956) who worked with Aloe also appear to be in agreement with this theory.

It was the aim of the present work to investigate the time factor of the effect of day length on morphogenesis, to establish the dependence of the effect of treatment on ontogeny, to demonstrate the role of the phase of shoot apex development at the beginning of the photoperiodic treatment and to examine the further course of development after photoperiodic treatment.

Methods

The work was carried out on the Niva spring-wheat. The technique of photoperiodic field experiments was the same as that described in the previous paper (Blum and Kříž 1956). In order to preclude any considerable changes in day length which represented control conditions during the experiments the sowing was carried out rather late during the year.

Table 1. Design of the experiment and position of the eight-hour day

Year sowing	Shoot emergence	Eight-hour day
1956 April 24	May 3	7 : 15 a.m. to 3 : 15 p.m.
1957 April 26	May 2	7 : 00 a.m. to 3 : 00 p.m.
1958 April 23	May 3	7 : 30 a.m. to 3 : 30 p.m.
1959 May 4	May 11	8 : 00 a.m. to 4 : 00 p.m.

The experiments were set up according to the classical arrangement (Hoňavka et al. 1956). The entire experiment was divided into a number of variants with different beginnings of the photoperiodic treatment; the time intervals between adjacent variants were not the same and are shown in Tables 2—5 together with the results of individual experiments.

The phase of the shoot apex was determined with the aid of a scale (Plate). The number of plants in a sample (3 to 10) increased with the development of the shoot apex. Morphological analyses of all plants (30 to 50 in each variant) were carried out either after reaching maturity or after termination of stalk growth (in 1959). The analyses were performed separately in the main stalks which had been marked in the stand. The results of analyses could then be compared with analyses of shoot apexes carried out only in the main stalks. The tables show the occurrence of plants departing from the norm. The upper limit of the norm was considered as the upper limit of a given feature in the control.

Acknowledgment:

The author is indebted to A. Martinovská and J. Kříž for technical assistance in carrying out the experiments.

Results

It follows from Tables 2—5 that the distribution of abnormities according to the time of treatment is analogous during the individual repetitions of the experiment. A particularly marked sequence of the maxima of occurrence of individual abnormities can be observed. Differences between individual years with different weather exist with respect to the absolute location of these maxima according to the time of photoperiodic treatment and in the dispersion around the maximum. There are also differences in the intensity of incidence of individual deviations during repetitions of the experiment.

The first abnormity to be observed during all repetitions is an abnormally large number of spikelets in the spike. This abnormity is most marked in the variants affected during the time when the shoot apex was still quite vegetative and possessed but a few leaf primordia (Plate II, Fig. 1). The excessive spikelets were formed only after the termination of treatment although a larger number of initiated spikelets could be observed even during prolonged photoperiodic influence (Plate II, Fig. 2a, b). The acropetal initiation of spike internodes continued then after the termination of the treatment until the upper internodes became differentiated into florets of the terminal spikelet.

Another abnormity caused by the short-day régime is branching. This abnormity was most frequent in the variants transferred to a short day during the growth in length of the shoot

Table 2. Percentage of abnormities in individual experimental variants in 1956

Number of 8-hour days	Age in days at the time of treatment	Shoot apex phase at the time of treatment	Increased number of spikelets (over 20)	Branching	Bract development		Spike with elongated internodes
					perfect	imperfect	
48	4—51	$1_{(1)}$*—2	**100**	—	—	—	—
45	7-51	$1_{(1)}$—2	**98**	—	—	—	—
42	10—51	$1_{(2)}$—3	**100**	—	—	—	—
39	13—51	$1_{(4)}$—5	39	6	52	—	—
36	16—51	$1_{(6)}$—5	46	**19**	37	—	—
33	19—51	1·5—5	25	6	60	32	—
30	22—51	2—6	12	8	**73**	36	12
27	25—51	3—6	—	—	20	**54**	38
24	28—51	5—7	—	—	2	7	**65**
21	31—51	6—7	—	—	2	—	28
18	34—51	6—7	—	—	—	—	6
15	37—51	7—7	—	—	—	—	4
12	40—51	7—7	—	—	—	—	—
9	43—51	7—7	—	—	—	—	—
6	46—51	7—7	—	—	—	—	—
3	49—51	7—7	—	—	—	—	—

* N.B. Number of leaf primordia on the shoot apex shown in parentheses.

Table 3. Percentage of abnormities in individual experimental variants in 1957

Number of 8-hour days	Age in days at the time of treatment	Shoot apex phase at the time of treatment	Increased number of spikelets (over 21)	Branching	Bract develcpment		Spike with elongated internodes
					perfect	imperfect	
51	1—51	1—2	73	—	4	—	—
48	4—51	1—2	50	—	9	—	—
45	7—51	1—2	**86**	—	18	—	—
42	10—51	1—2	60	—	—	—	—
39	13—51	1—3	66	—	4	—	—
36	16—51	1—5	15	12	35	—	—
33	19—51	2—5	3	**30**	93	3	—
30	22—51	2—6	4	15	**96**	19	4
27	25—51	2—7	—	—	67	26	11
24	28—51	3—7	—	—	7	**48**	44
21	31—51	4—7	—	—	7	21	**72**
18	34—51	5—7	—	—	—	3	50
15	37—51	6—7	—	—	—	—	9
12	40—51	7—7	—	—	—	—	7

Table 4. Percentage of abnormities in individual experimental variants in 1958

Number of 8-hour days	Age in days at the time of treatment	Shoot apex phase at the time of treatment	Increased number of spikelets (over 21)	Branching	Bract development		Spike with elongated internodes
					perfect	imperfect	
48	6—54	$1_{(2)}$*—2	30	—	—	—	—
42	13—54	$1_{(2)}$—4	28	—	—	—	11
41	14—54	$1_{(3)}$—4	**83**	—	24	—	3
40	15—54	$1_{(6)}$—5	**89**	—	19	—	6
39	16—54	$1_{(6)}$—5c	77	6	40	3	23
37	18—54	$1_{(7)}$—5	44	39	82	5	7
36	19—54	1·5—5	26	23	65	13	18
35	20—54	1·5—5	20	**40**	**95**	5	20
34	21—54	2—6	2	33	**97**	21	13
33	22—54	2—6	3	4	**93**	25	10
32	23—54	2—6	—	12	**91**	44	6
31	24—54	2—6	4	9	**91**	57	5
30	25—54	2—7	3	3	80	59	3
29	26—54	3—7	—	—	37	75	—
28	27—54	3—7	—	—	23	**80**	12
27	28—54	4—7	—	—	—	**85**	25
26	29—54	4—7	—	—	—	**82**	18
25	30—54	4—7	—	—	—	59	35
24	31—54	5—7	—	—	—	71	39
23	32—54	5—7	—	—	—	19	**54**
20	35—54	5—7	—	—	—	3	**57**

Table 5. Percentage of abnormities in individual experimental variants in 1959

Number of 8-hour days	Age in days at the time of treatment	Shoot apex phase at the time of treatment	Increased number of spikelets (over 20)	Branching	Bract development		Spike with elongated internodes
					perfect	imperfect	
50	2—51	$1_{(1)}$*—2	2	—	—	—	—
47	5—51	$1_{(2)}$—2	**20**	—	—	—	—
44	8—51	$1_{(2)}$—3	2	—	—	—	—
41	11—51	$1_{(3)}$—5	2	—	4	—	4
38	14—51	$1_{(5)}$—5	—	**22**	42	4	28
35	17—51	2—5	—	5	**60**	30	14
32	20—51	2—6	—	—	20	**72**	7
29	23—51	3—7	—	—	5	30	62
26	26—51	4—7	—	—	2	10	**88**
23	29—51''	5—7	—	—	—	2	27
17	35—51	7—7	—	—	—	—	3
11	41—51	7—7	—	—	—	—	—

* N.B. Number of leaf primordia on the shoot apex shown in parentheses.

apex (Plate II, Fig. 3), or possibly at the beginning of differentiation of spikelet primordia (in 1957). The branching took place particularly in the lower third of the spike. Branched spikelets were frequently imperfectly developed (Plate II, Fig 4a) and dried during maturation. We could never succeed in detecting even a trace of branching during the usual duration of photoperiodic treatment; it was found only after the termination of treatment. These spikelets resembled an embryonal spike and bore a much higher number of florets than could be ascertained later during morphological analyses (Plate II, Fig. 4b).

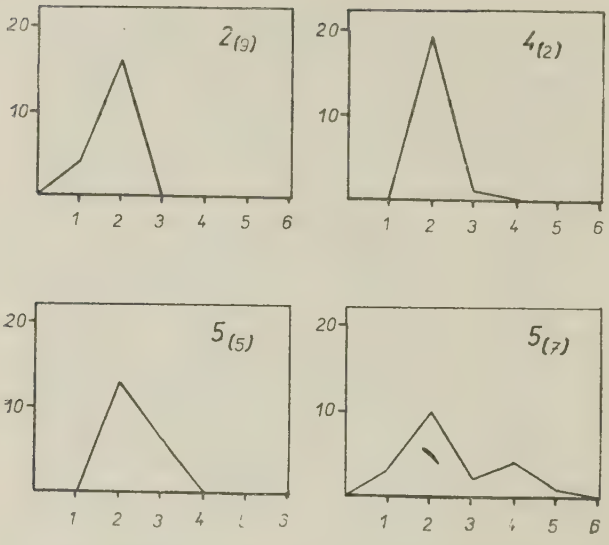
Repetition of experiments on the same experimental lot confirmed the marked dependence of the formation of the two above-mentioned abnormities on growth conditions. In a large number of spikelets the aggravated growth conditions were reflected not only in the smaller representation and in the less distinct abnormity but also in a shift of the maxima toward later and thus also shorter photoperiodic treatment. The effect of growth conditions was most marked in the plants which were left on the short-day régime for a longer time than indicated in the tables.

Another type of abnormity is represented by the development of bracts below one or two lowermost spikelets. The bract either resembles a leaf or develops imperfectly and exhibits very unsual shapes. The first of these abnormities occurs mostly in experimental variants affected during the time of spikelet initiation (Plate III, Fig. 5a). The second abnormity is most frequent with variants which were affected during the time of floret differentiation (Plate III, Fig. 5b), or immediately preceding it. Although these deviations may differ at first sight there is but a quantitative difference between them. A gradual transition between the two exists (Plate III, Fig. 6). The leaf blade is the first to be reduced (Fig. 6b), followed by the sheath (Fig. 6c), the last being the peripheral parts of the sheath, or possibly a narrow strip along the central rib. One of the supporting bracts is thus represented by 2 or 3 primordia (Fig. 6d, e) and in the tables is marked as an imperfectly developed bract. These primordia can reach a length of several cm. to several mm. and finally pass over into a groove which is quite marked occasionally even in control plants (Fig. 6f). The spikelet in the axil of a developed bract is frequently branched, particularly in the axil of bracts with a lamina (Plate IV, Fig. 7). The axillary spikelet of an imperfectly developed bract is always unbranched. The development of the axillary spikelet is markedly suppressed, the more so the more fuller developed is the bract. The length of the basal spike internode is also related to the development of the bract.

The bract primordia could be detected only after termination of the photoperiodic treatment. The embryonal bract was characterized by the fact that it was very early differentiated into a sheath and a lamina (Plate IV, Fig. 8). The high level of the ligula indicated that the lamina was inhibited more than the sheath. The primordia of an undeveloped bract could be detected still later, i.e. immediately before earing (Plate IV, Fig. 9). The bract is formed from a leaf primordium at the base of an embryonal spike. This leaf primordium, even under normal conditions, reaches a greater length than the following primordia, occasionally as much as 0·3 mm. In the axil of this leaf primordium there is a spikelet primordium which lags behind the remaining spikelets even under normal conditions. This difference in the development of central and lower spikelets is still increased in the corresponding variants following photoperiodic treatment (Plate IV, Fig. 10).

The growth conditions were little reflected in the development of the bract itself but were quite pronounced in the development of the axillary spikelet. The prolongation of the photoperiodic treatment until the time of earing with some of the plants had no effect on the occurrence or on the formation of the supporting bract. The development of the axillary spikelet was, however, greater after a prolonged inhibition period. In plants in which the beginning of the treatment corresponded in Tables 2—5 to the maximum of formation of bracts, after longer treatment the axillary spikelet represented a bud with several leaves (transformed glumes or lemmas) and with a branched inhibited spikelet (Plate IV, Fig. 11). After a sufficiently long treatment period and under good growth conditions these leaves underwent growth, the interposed internodes were elongated and in some cases a secondary spike appeared (Plate IV, Fig. 12).

The last abnormity to be observed was an elongation of the internodes of the rachis which again only affected the lowest internodes. This abnormity was formed mostly after short-day inhibition at the time of initiation of stamina (Plate IV, Fig. 8 and 10). The formation of this abnormity could be detected only before the time of earing, i.e. a longer time after the termination of the photoperiodic treatment.



Text-Fig. 1. Examples of variability of development of the shoot apex and of tillers in comparison with the developmental phase of the main stalk (upper right). x — developmental phase of the shoot apex of tillers; y — frequency of this phase.

It follows from the distribution of the maxima of occurrence of individual abnormities that identical abnormities were obtained by treatment at different ages in days of plants. These differences are in agreement with the different course of development of the shoot apex of the cont rol due to the differences in weather during the individual repetitions of the experiment.

Under the short-day conditions the development of the shoct apex was also influenced by the weather. It is apparently for this reason that the variants with a corresponding phase of the shoot apex at the beginning of treatment were actually in different shoot apex phases at the end of treatment in different years.

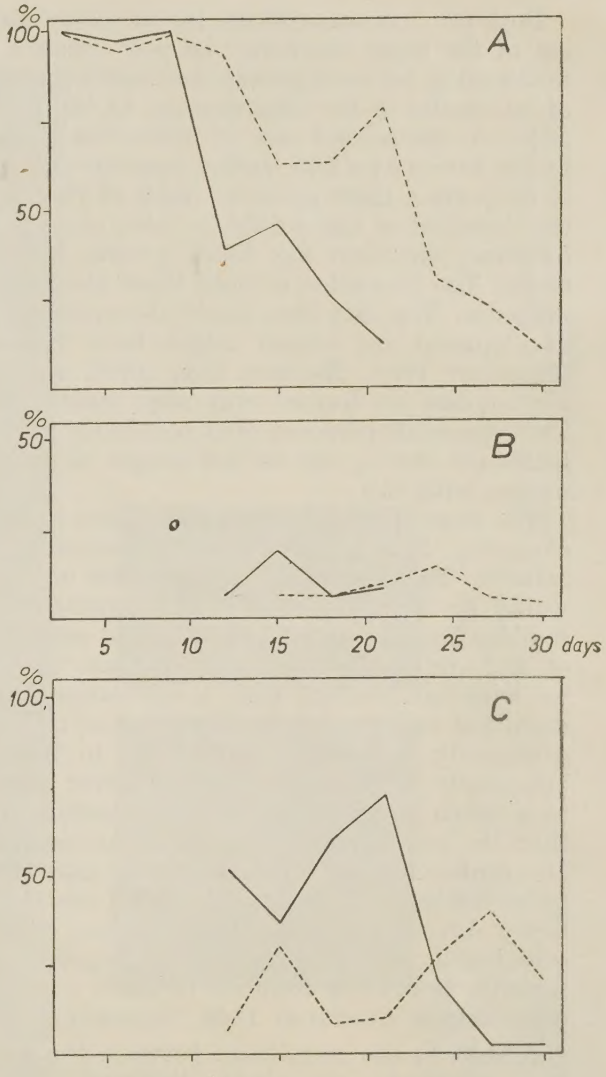
The results shown in the tables indicate a relationship between the type of abnormity and the developmental stage of the shoot apex immediately prior to the photoperiodic treatment.

In the tillers the entire course of differentiation of the shoot apex is delayed in comparison with the main stalk (Text-fig. 1); therefore, after certain photoperiodic treatment, the tillers display a representation of different types of abnormities corresponding to an earlier phase of development of the shoot apex in the main stalks. The Text-fig. 2 gives an example of the differences in distribution of abnormities between primary and secondary spikes. The curves show that the abnormities exhibit shifted maxima and also that the percentage of abnormal spikes is lower. Part of the tillers develop quite normally after the termination of photoperiodic treatment.

The relationship between the type of abnormity and the treatment applied at a given developmental stage of the shoot apex results in the fact that individual variants display a number of other abnormities in addition to the prevalent one. This morphological variability is due to a developmental variability of the plants in the experimental variant prior to treatment. Minute differences at the beginning of the photoperiodic treatment result, under the conditions of retarded development, in a considerably different morphogenesis.

Discussion

The present work has shown that the developmental phase of the shoot apex at the time of the beginning of photoperiodic treatment is of decisive significance for the formation of a definite type of abnormity. The relationship between the developmental phase of the shoot apex at the beginning



Text-Fig. 2. Occurrence of abnormitiesof the main shoot (continuous line) and of tillers (brokenf line). *x* — age of plants at the time of photoperiodic treatment (experiment of 1956). A — increased number of spikelets, B — branching of spikelets, C — development of bracts.

of treatment and the resulting morphogenesis was identical during all repetitions of the experiment and was also in agreement with the results obtained in an earlier investigation (Blum and Kříž 1956).

If the treatment took place at a time when the shoot apex was completely vegetative only an increased number of spikelets could be observed. Where other authors describing this abnormity devoted their attention to the effect of time of the short-day treatment it may be seen that the short day exerts its influence either immediately after shoot emergence (Sapegin 1938, Lobov 1946, Kornilov 1952, Fedorov 1954, Du-Zho-Fu 1958a, b) or at least before the differentiation of spikelets (Hänsel 1951, Alleweldt 1958, Batygin 1959).

Branched spikes were formed in the experiments described here most frequently following treatment during the longitudinal growth of the shoot apex, less so during the beginning of spikelet differentiation. A relationship between treatment during spikelet initiation and the formation of branched spikelets was found by Bassarskaya (1946), Du-Zho-Fu (1958a, b) and Batygin (1959). Wislocka (1955, Wislocka et al. 1957) found treatment immediately before spikelet initiation most suitable for obtaining branched spikes. A similar relationship was found by Novikov (1953) with winter rye. All these papers indicate that branching takes place before floret differentiation, only Surkov (1959) considers the florets on branched rye spikelets as modified stamina.

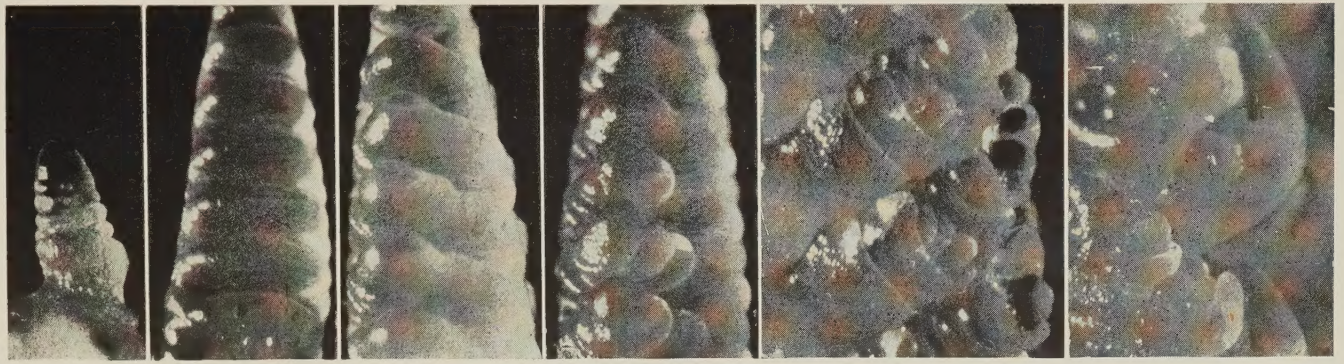
Both the first abnormities, the increased number of spikelets and branching, are of the same character. In both cases a shift in the interference of the relationship between growth and development results in an increased number of internodes in the inflorescence, in the first case of internodes of the same order, in the second case of internodes of subsequent orders. The short day exerts here a two-fold, rather opposite influence on morphogenetic processes. It supports a more powerful habit of the spike via developmental inhibition, the duration of this inhibition also playing a role here. In addition to this, however, the short day limits growth by shortening the time of photosynthesis. The formation of both these abnormities depends on the predominant influence. The fact that these abnormities formed during an early phase of development are absent might have led some authors (NIKITENKO 1951, KORNILOV 1952, MATHON 1954, 1955, and others) to the opinion that the abnormities are formed only after treatment at the end of the light stage. The repeatedly demonstrated possibility of the formation of abnormities on treatment during the earliest stages of development is in apparent contradiction with this.

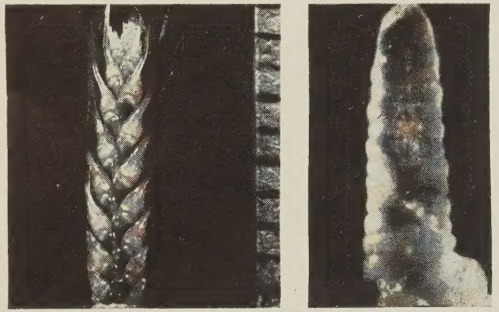
The next abnormity, the development of a bract, is of an entirely different character. It is a shift of the correlation between the bract and the axillary spikelet. This results in the formation of a pair of organs, of which only one would be formed under normal conditions. The observed phenomenon resembles DOSTÁL'S (1959) experiments with the correlation between the growth of leaf and a vegetative axillary bud. In the case described here, however, we were not dealing with a correlation between the leaf and the axillary shoot but rather with the formation of the leaf and the development of ontogenetically more advanced organs in the axillary spikelet. The sheath is apparently inhibited by the generative development of the axillary spikelet to a much lesser degree than the lamina. It should be noted at this point that the normal leaf organs in Gramineae spikes (glumes and lemmas) are also represented by a sheath (PHILLIPSON 1934). The sheath margins appear to be the least inhibited, this giving rise to the strange appearance of imperfectly developed bracts. The division of a bract into two primordia and occasionally also an abnormal elongation of the glumes following photoperiodic treatment resulted in some cases in an erroneous interchange of these organs (NOGUCHI 1929, ALLEWELDT 1958, DU-ZHO-FU 1958a).

A shift in the correlation between the growth of bracts and the development of spikelets was always observed in the so-called labile primordia (PURVIS and GREGORY 1937) which, even under normal conditions, differ in their development from the rest of the spikelets. The lagging spikelets are most easily affected by photoperiodic inhibition. The present work as well as the papers of DU-ZHO-FU (1958a, b) and ALLEWELDT (1958) describe the most frequent spikes with a supporting bract following treatment at the time of differentiation or immediately prior to it (BLUM and KŘÍŽ 1956). According to the results of PETR (1958) incompletely developed bracts appear after treatment at the beginning of spikelet differentiation, according to DU-ZHO-FU (1958a, b) they are formed after treatment during the phase of spikelet differentiation, according to our results, however, after treatment toward the end of spikelet differentiation or at the beginning of differentiation of florets

Scale of shoot apex development (vertical dimension of photograph = 1 mm.). Progressive formation of ontogenetically more advanced organs.

Stage 1. Stage 2. Stage 3. Stage 4. Stage 5. Stage 6.





Stage 7. Stage 1-5.

1 — Initiation of leaves
2 — Initiation of spikelet primordia
3 — Initiation of florets on flower primordia
4 — Differentiation of glumes and lemmas on flower primordia
5 — Initiation of stamens of flower primordia
6 — Differentiation of anthers into anther lobes
7 — Formation of stigmas

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J. Brčák: Transmission of *Marmor cichorii* Kvíčala to *Chenopodium giganteum* Don.

J. Krekule: The Effect of Photoperiodic Regime of Vernalization of Winter Wheat

K. B. Deshpande: Antagonism Between *Rhizoctonia solani* Kühn and Certain Soil Saprophytes

J. Uhlík: A Method of Rapid Examination of Meiosis in Fruit Trees

J. Uhlík: The Cytological Evaluation of Some Pear-Sorts Cultivated in Czechoslovakia

J. Kloz: The Use of the Quantitative Ring Precipitation Reaction for the Determination of Cross Reactions

F. Seidlová-Blumová: On the Determination of the Course and Termination of Photoperiodic Sensitivity in Spring Wheat *(Triticum vulgare)*

J. Vacke: Wheat Dwarf Virus Disease

B. Němec: Über mixoploide Wurzelspitzen